Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL *of* CANADA

VOLUME 6 FEBRUARY, 1932 NUMBER 2

THE MILLING AND BAKING QUALITY OF FROSTED WHEAT OF THE 1928 CROP[1]

BY W. F. GEDDES[2], J. G. MALLOCH[3] AND R. K. LARMOUR[4]

Abstract

Owing to limited rainfall following germination, combined with late heavy frosts, a large portion of the 1928 wheat crop of Western Canada contained many types of frost damage together with immature and green kernels. A survey of the crop was made in the three laboratories collaborating with the Associate Committee on Grain Research with the dual object of studying the Canadian grading system as applied to frosted wheat and of securing information on the relative effects of green, immature, and frosted kernels on milling and baking quality. The study is based on 228 samples grading from No. 1 Northern to No. 6.

Physical examinations showed that the percentage of sound kernels progressively decreased with a decrease in grade, with a greater relative increase in the percentage of "heavy damage" in the lower grades. Test weight per bushel also decreased. Partial correlations showed that individually the various forms of damage had only a slight effect on reducing weight per bushel, heavily frosted and immature kernels having a greater influence than bran frosted kernels.

On a regrading of the samples after storage over winter, 83.3% of the samples were unchanged in grade while 13.2% received a higher grade.

The mean total flour yield decreased with grade, the variability in yield being much higher within the commercial grades. Owing to the tough and fibrous nature of the middlings there was approximately a 20% increase in the time required to mill a sample of No. 5 or No. 6 wheat as compared with the statutory grades. Bran frost, heavy frost, and immature kernels are negatively correlated with flour yield and are of approximately equal importance in their effects. Weight per measured bushel and the percentage of total sound kernels are the best single indices of flour yield.

Baking quality was determined in the three laboratories using either a 55% patent or a straight grade flour and baking according to the simple, bromate, malt and blend formulas. While the simple formula gave incongruous results all the others revealed that the average baking quality as measured by loaf volume, crumb color and texture decreased with grade except in the instance of grade No. 4 which was superior to No. 3 Northern. Absorption markedly increased in the lower grades. The straight grade and patent flours gave the same relative results when baked by either the simple or the bromate formula.

[1] *Manuscript received December 18, 1931.*

Contribution from the Department of Agricultural Chemistry, University of Manitoba, Winnipeg; Department of Field Crops, University of Alberta, Edmonton; and Department of Chemistry (supported by the Saskatchewan Agricultural Research Foundation), University of Saskatchewan, Saskatoon; with financial assistance from the National Research Council of Canada. Issued as Paper No. 29 of the Associate Committee on Grain Research.

[2] *Professor of Agricultural Chemistry, University of Manitoba, Winnipeg, Manitoba.*

[3] *Research Assistant, Associate Committee on Grain Research, University of Alberta, Edmonton, Alberta.*

[4] *Associate Professor of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan.*

The average responses to the differential baking tests also decreased with wheat grade, the magnitude of the individual responses being correlated with protein content. Partial correlations calculated for the response to bromate showed that both protein content and percentage of sound kernels are positively correlated with this variable.

The variability in baking quality within each grade increased with decreasing grade, owing in part to variations in the percentage of sound kernels, but chiefly to variations in protein content. Protein content of wheat is the best single measure of baking quality within each grade, but is not a reliable measure when comparisons are made between grades, owing to differences in protein quality. Partial correlations showed, as in the instance of milling quality, that the various classes of visible damage need not be considered individually with regard to their effects on baking quality.

The results of this study indicate that weight per measured bushel and either the percentage of total sound or hard red vitreous kernels could be used to advantage as grading factors in the commercial grades. It is concluded that the grading system in use in 1928 was applied in an efficient manner and gave a satisfactory indication of the relative quality of the various grades with the exception of the anomalous relationship between No. 3 Northern and No. 4.

The variability in baking quality within grades is excessively high, particularly in the lower grades. Although variations in protein content are chiefly responsible for the lack of uniformity within grades, some improvement may be effected by restricting the variability in the percentage of total sound or vitreous kernels allowable in the various grades. The revisions in the Canada Grain Act instituted in 1930 during the progress of the investigation, are in line with the results of this study and may be expected to bring about a greater uniformity in grade characteristics.

Introduction

In the extensive wheat growing areas of Western Canada the crop is grown under such a wide range of soil and climatic conditions that frequently a portion of the wheat marketed contains green, immature, and frosted kernels. Normally such wheat does not constitute a very large proportion of the total crop, but in the year 1928 a combination of conditions prevailed over the prairie provinces which resulted in a wheat crop of low average quality. Owing to a period of limited rainfall following seeding, germination was not uniform; severe and general frosts occurred in the third week of August. As a result of these two conditions, there were throughout a large proportion of the crop many types of frost damage, combined with green and immature kernels. While the largest wheat crop in the history of the West was produced in that year, the damage was so widespread that 59% of the cars inspected by the Western Division was graded into the commercial grades. As there was no rust epidemic that season, the degrading of the wheat was due principally to frost injury, and resulted in extensive economic losses to the wheat producers of Western Canada.

The widespread occurrence of frosted wheat containing varying percentages of frosted, immature and green kernels provided an excellent opportunity for a study of the influence of these types of damage on milling and baking quality, and in view of its general importance, an extensive investigation of different phases of the problem was undertaken by the Associate Committee on Grain Research as one of its collaborative projects.

In addition to annual surveys of the milling and baking quality of commercial wheat containing frost damaged kernels, the plan of investigation included a study of the quality of wheat artificially frozen at different stages of maturity

in comparison with normal samples grown under the same soil and climatic conditions. Another phase of the problem involved a study of the physico-chemical and chemical changes taking place during maturation of the wheat kernel and the influence of freezing on the course of these changes.

The present report covers the results obtained in a survey of the 1928 crop which was undertaken with the dual object of studying the grading system as applied to frosted wheat, and of securing information on the relative effects of the presence of green, immature and frosted kernels on milling and baking quality.

Experimental

The material used in this investigation consisted of 228 samples of hard red spring wheat grown in various districts throughout the three prairie provinces in the crop year of 1928. Of this number, 14 were composites made up from samples taken for grading and supplied by the Western Grain Inspection division, while the remainder were collected for the Committee by the Canadian Co-operative Wheat Producers Limited through their elevator agents. Fifty-two of the samples originated in Alberta, 138 in Saskatchewan and 24 in Manitoba.

The samples were cleaned, divided into subsamples, using a Boerner sampler, and forwarded to the cereal research laboratories of the Department of Field Crops, University of Alberta; the Department of Chemistry, University of Saskatchewan; and the Department of Agricultural Chemistry, University of Manitoba, for determinations of milling and baking quality. Subsamples were also supplied to the Western Grain Inspection Branch at Winnipeg for grading, and to the Grain Research Laboratory of the Board of Grain Commissioners, Winnipeg, where the physical classification of the kernels was carried out.

I. Physical Characteristics of the Grades

Since the problem was to relate the grading of wheat to its actual milling and baking quality, it seemed logical to examine first the physical characteristics of the wheat in the various grades. Fifty grams of wheat was accordingly weighed from each sample and hand picked into the following classes:

(a) Vitreous—well-matured kernels free from frost damage and starchy spots.

(b) Piebald—well-matured kernels free from frost damage, but having starchy spots.

(c) Starchy—well-matured kernels free from frost damage, but completely starchy.

(d) Bran Frost—kernels showing wrinkling of the bran which did not extend into the crease.

(e) Heavy Frost—all kernels showing frost damage not included in (d).

(f) Immature—fully formed kernels having a dark color; usually referred to as "bronzy" or "pink" kernels.

(g) Green—kernels having a decided green color; usually shrivelled.

After classification, the separates were weighed and the percentage of each calculated. In the instance of some samples there were small quantities of broken kernels, foreign matter, etc., which it was impossible to classify into any of the above groups and these were placed in a separate class.

The physical examinations must be considered as approximate. They were however carried out on the entire series of samples by one individual with frequent rechecking, so that the results may be regarded as fairly consistent.

The weight per measured bushel was determined in one of the collaborating laboratories. For this purpose a container of slightly more than the capacity of the test bucket was filled to overflowing, the excess stroked off with a round strike and the wheat poured into a Cox funnel. This was placed on top of the test bucket and the grain was allowed to run in. After stroking off as before, the weight was taken using a beam of the standard pattern. Since the samples had been stored in the laboratory for some time before these measurements were made, and had dried to a moisture content of approximately 8%, the values obtained for weight per bushel were considerably higher than they would have been at a normal moisture content of 13 to 14%. The samples, however, were quite uniform in moisture content and the weights are thus comparable.

The means for the different classes of kernels and weight per measured bushel are arranged according to grade in Table I. Since only 8 samples graded No. 1 Northern, the results for grades No. 1 Northern and No. 2 Northern were combined. In this table the means for the nondescript class, which was made up of broken kernels etc., have been omitted, thus accounting for the fact that a summation of the percentages of the different classes of kernels does not total 100. For the purpose of statistical study the percentages of vitreous, piebald, and starchy kernels have been grouped together as "Total Sound" and the percentages of heavily frosted, green and immature kernels as "Heavy Damage", and these are also recorded in the table.

Before discussing the relation between physical characteristics and wheat grade it is necessary to consider briefly the Canadian grading system. The regulations governing the statutory grades of red spring wheat are specified in the Canada Grain Act. In 1930, during the progress of this investigation, the definitions were materially amended. The most important changes involved the definition of No. 4 as a statutory grade and an increase in the percentage of hard vitreous kernels required in the different grades. Table II gives the definitions governing the statutory grades in 1925 and also the revisions of 1930. The samples in this study were graded according to the definitions of 1925 and as No. 4 was then a commercial grade it will be referred to as such throughout this report.

The characteristics of the commercial grades No. 4, No. 5, No. 6. and Feed are, however, not laid down in the Canada Grain Act, since the predominating forms of damage vary from year to year. The standards for these grades are established each year by the Grain Standards Board after the crop movement has begun and the prevailing types of damage ascertained. Wheat not eligible for the statutory grades is thus graded into one or other of the commercial grades, by comparison with standard samples depending principally on the type and extent of the prevailing forms of damage.

TABLE I

MEANS, STANDARD DEVIATIONS AND Z VALUES FOR WEIGHT PER MEASURED BUSHEL, AND CLASSES OF KERNELS

Grade	No. of samples	Weight per bush.		Vitreous	Piebald	Starchy	Total sound (1)		Bran frost (2)		Heavy frost (3)		Immature (4)		Green (5)		H'vy damage (7)	
		Mean lb.	S.D. lb.	Mean %	Mean %	Mean %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %
1° and 2°	52	64.7	1.3	79.2	5.8	0.4	85.5	6.0	1.9	2.3	0.0	0.0	9.1	4.7	0.3	0.5	9.4	4.7
3°	38	64.3	1.0	64.9	7.7	1.6	74.2	9.9	5.3	4.7	0.4	1.7	15.5	9.5	0.9	0.7	16.8	9.4
4	51	63.7	1.2	50.1	6.4	2.0	58.6	15.2	15.9	11.2	4.2	6.6	15.3	8.3	1.5	1.5	21.1	9.5
5	48	62.5	1.4	24.0	6.2	0.5	30.8	16.5	28.8	17.0	13.2	14.7	19.9	12.8	2.9	3.0	36.0	15.3
6	39	61.3	1.6	16.2	1.4	1.0	18.6	11.1	28.5	15.7	24.9	16.3	18.0	13.5	5.7	5.4	48.5	14.7
Z		1.9309					2.7102		2.5144		1.9060		1.0376		1.6020			
5% Pt.		0.4403					0.4403		0.4403		0.4403		0.4403		0.4403			

NOTE:—*1° = No. 1 Northern; 2° = No. 2 Northern; 3° = No. 3 Northern; 4 = No. 4 Wheat; 5 = No. 5 Wheat; 6 = No. 6 Wheat.*

TABLE II

STATUTORY GRADES OF WESTERN GRAIN—RED SPRING WHEAT

Definitions according to Canada Grain Act 1925*

Number and name of grade	Minimum weight per bushel in lb.	Variety of grain	Percentage of hard vitreous kernels	Standard of quality	Foreign material other than dockage
No. 1 Manitoba Hard	62	Marquis or equal to Marquis	75	Sound	Well cleaned
No. 1 Manitoba Northern	60	Marquis or equal to Marquis	60	Well matured, practically free from damaged kernels	Well cleaned and practically free from foreign grains
No. 2 Manitoba Northern	58	Marquis or equal to Marquis	45	Reasonably sound	Reasonably clean
Or	60	Soft varieties of Red Spring wheat	60	Sound	Reasonably clean, may contain Amber or Red Durum singly or in combination up to 1%
No. 3 Manitoba Northern	57	Red spring wheat varieties fair milling quality		Reasonably sound	Reasonably clean, may contain Amber or Red Durum wheat, singly or in combination up to 3%

Definitions according to Canada Grain Act 1930, Schedule 1.

Number and name of grade	Minimum weight per bushel in lb.	Variety of grain	Percentage by weight of hard vitreous kernels	Standard of quality	Foreign material other than dockage		Wheat of other classes	
					Matter other than cereal grains	Total including cereal grains %	Durums %	Total including Durum %
No. 1 Manitoba Hard	62	Marquis or equal to Marquis	80	Sound and well matured	Free	Free		
No. 1 Manitoba Northern	60	Marquis or equal to Marquis	65	Well matured, practically free from damaged kernels	Free	Practically free	Practically free	1
No. 2 Manitoba Northern	58	Red spring wheat of good milling quality	50	Reasonably well matured, reasonably free from damaged kernels	Free	About 1%	1	3
No. 3 Manitoba Northern	57	Red spring wheat of fair milling quality	25	Reasonably well matured, reasonably free from damaged kernels	Reasonably free	About 2%	3	10
No. 4 Manitoba Northern	57	Red spring wheat		Reasonably well matured, but excluded from preceding grades on account of frosted or otherwise damaged kernels	Reasonably free	About 2½%	4	10
Or	55	Red spring wheat		Rusted or shrunken but otherwise reasonably sound	Reasonably free	About 2½%	4	10

**Arranged in table form by T. J. Harrison, Assistant Grain Commissioner for Manitoba.*

The setting up of standards for the commercial grades of the 1928 crop presented a difficult problem owing to the existence of two types of damaged kernels. As a consequence of uneven germination there was an unusually large percentage of immature and green kernels in the threshed grain, while the general occurrence of late heavy frosts, combined with uneven growth, resulted in many types of frost damage in grain harvested from the one field. In grading such wheat it was therefore necessary to balance one type of damage against another.

The mean values for weight per bushel and classes of kernels recorded in Table I and graphically represented in Fig. 1 show a definite gradation from the higher to the lower grades and it is important, therefore, to have some measure of the significance of this gradation or trend. Since the grade numbers obviously do not represent numerical differences between the grades it is illogical to calculate correlation coefficients for the relations between the means for the physical classes and the grade numbers. The best measure of the significance of the trend is given therefore by the significance of the variance contributed by the differences between the means for the grades as compared to the variance between the samples within the grades. This procedure is identical with that of testing the significance of the correlation ratio. The analysis for each physical class is carried out as in Table III for weight per bushel.

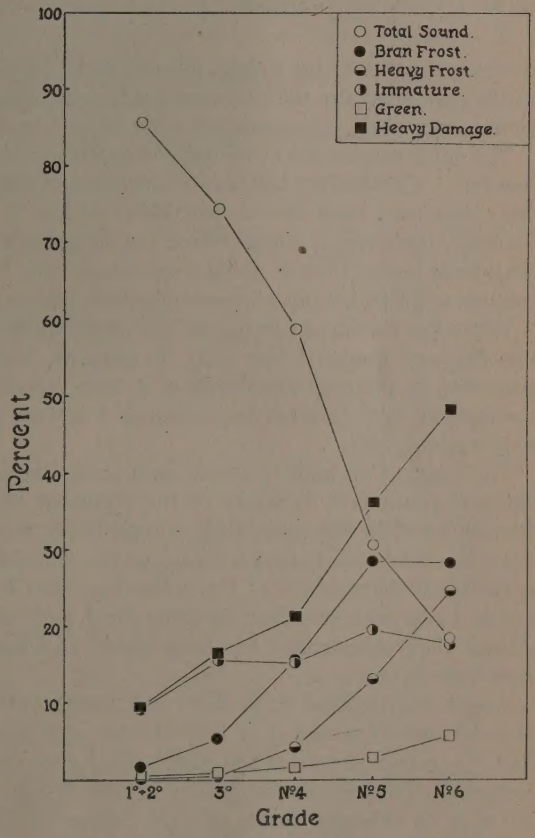


FIG. 1. *Showing the mean percentage of the different classes of kernels according to wheat grade.*

For 4 and 223 degrees of freedom the 5% point for Z is 0.4403 so that the variance between grades is very significantly higher than that within grades. The Z values representing differences between the grades for the means of the physical classes are given with their respective 5% points at the foot of Table I. In every instance the Z values exceed the 5% point, and hence the differences

TABLE III

COMPARISON OF VARIANCE BETWEEN AND WITHIN GRADES FOR WEIGHT PER MEASURED BUSHEL

Variance due to	Sum of squares	Degrees of freedom	Variance	1/2 log e	Z
Between grades	346.06	4	86.52	2.2303	1.9309
Within grades	405.20	223	1.82	0.2994	
Total	751.26				

between the means for weight per measured bushel and the various classes of kernels recorded for the different grades are significant. In other words the grades are clearly differentiated with respect to their physical characteristics.

The total percentage of damaged kernels increases markedly as the grade is lowered. Considering the individual forms of damage, the percentage of bran frost increases from No. 1 Northern to No. 5, and then decreases. This decrease, however, is compensated by the greater relative increase in the percentage of heavy frost in going from Grade No. 5 to Grade No. 6. The percentage of green kernels increases regularly with a decrease in grade.

While the significant trends in the mean values for the classes of kernels in the different grades imply that, in general, the present system of grading according to physical classification is being applied in a satisfactory manner, the grading system must be examined from the point of view of uniformity within each grade.

The range of variability within each grade has been measured for each of the physical characters in terms of the standard deviation. While there is no definite trend in the variability of weight per bushel for the different grades, there is a fairly consistent increase in the variability of the classes of kernels as the grade decreases from No. 1 Northern and 2 Northern to No. 5. Similar trends have been observed in flour yield and baking quality. In these instances their significance has been tested and this question will be discussed more fully later.

Simple correlations have been calculated between the various classes of kernels, and are recorded in Table IV for each grade, and for the entire series. The 5% points are stated at the foot of each section giving the correlation coefficients for a particular grade. These 5% points were determined by the use of R. A. Fisher's tables of t (3). Since all the coefficients involve more than 30 degrees of freedom the t value for the 5% point is approximately 2.0. Then from the formula for t,

$$t = \frac{r\sqrt{n}}{\sqrt{1-r^2}},$$

where n is the number of degrees of freedom, we obtain,

$$r = \frac{2}{\sqrt{n+4}}.$$

To facilitate the interpretation of the table the correlations which are statistically significant have been printed in italics.

In grades No. 1 Northern and No. 2 Northern the percentages of immature and bran frosted kernels, which are generally regarded as the less severe forms of damage, are chiefly responsible for the variation in the percentage of sound kernels. As the grade decreases the magnitude of the negative correlation between total sound and immature progressively decreases, and in grades No. 5 and No. 6 the percentages of heavily frosted kernels become a significant factor in causing a decrease in the percentage of sound kernels. This is indicative of a progressive change in the character of the samples going into each grade.

TABLE IV

SIMPLE CORRELATIONS BETWEEN CLASSES OF KERNELS

Grade	Class of kernel		Bran frost (2)	Heavy frost (3)	Immature (4)	Green (5)
1° and 2°	Total sound	(1)	− *.463*	+ .174	− *.848*	− .094
	Bran frost	(2)		− .092	+ .032	− .031
	Heavy frost	(3)			− .092	+ .021
	Immature	(4)				+ .048
	5% Pt. $r = .271$					
3°	Total sound	(1)	− .207	− .017	− *.839*	+ .165
	Bran frost	(2)		− .092	− .210	− *.327*
	Heavy frost	(3)			− .160	+ .014
	Immature	(4)				− .078
	5% Pt. $r = .316$					
No. 4	Total sound	(1)	− *.756*	− .257	− *.569*	− .057
	Bran frost	(2)		− .049	+ .151	− .148
	Heavy frost	(3)			− .229	− .241
	Immature	(4)				+ .212
	5% Pt. $r = .275$					
No. 5	Total sound	(1)	− *.568*	− *.398*	− .109	+ .156
	Bran frost	(2)		− .135	− *.344*	− *.300*
	Heavy frost	(3)			− *.397*	− *.307*
	Immature	(4)				+ *.313*
	5% Pt. $r = .283$					
No. 6	Total sound	(1)	− *.386*	− *.410*	+ .046	+ .104
	Bran frost	(2)		− .307	− *.441*	+ .105
	Heavy frost	(3)			− *.382*	− *.431*
	Immature	(4)				− .100
	5% Pt. $r = .312$					
Entire series	Total sound	(1)	− *.780*	− *.671*	− *.402*	− .439
	Bran frost	(2)		+ *.311*	+ .043	+ *.286*
	Heavy frost	(3)			− .065	+ *.147*
	Immature	(4)				+ *.188*
	5% Pt. $r = .132$					

It will also be observed that for grades No. 5 and No. 6 the percentage of bran frost is negatively correlated with immature kernels. For these grades heavy frost also is negatively correlated with immature and green kernels, indicating that one form of damage is being balanced against another in determining the grade.

TABLE V

SIMPLE, PARTIAL, AND MULTIPLE CORRELATION COEFFICIENTS INVOLVING WEIGHT PER MEASURED BUSHEL AND CLASSES OF KERNELS

	Simple correlation coefficients					Third order partial correlation coefficients
	Total sound (1)	Bran frost (2)	Heavy frost (3)	Immature (4)	Green (5)	
Weight per bushel (w)	+ .595	− .319	− .446	− .351	− .309	$r_{w.2.345}$ − .158
Total sound (1)		− .780	− .671	− .402	− .439	$r_{w.3.245}$ − .440
Bran frost (2)			+ .311	+ .043	+ .286	$r_{w.4.235}$ − .366
Heavy frost (3)				− .065	+ .147	$r_{w.5.234}$ − .159
Immature (4)					+ .188	
Green (5)						
	At 5% pt. $r = .132$					At 5% pt. $r = .133$

Multiple $R_{w.2345} = .625$ 5% pt. $= .320$

Relation Between Weight per Measured Bushel and Classes of Kernels

From the standpoint of wheat grading it is of interest to examine the relations between the physical characteristics of wheat that affect grading. As shown in Table V, there is a negative correlation between weight per bushel and all of the forms of damage and since "total sound" represents all of the remaining kernels after the damaged ones have been removed, there is a positive correlation between weight per bushel and "total sound". The partial correlations have been determined as shown in the table for weight per bushel with each of the forms of damage when all of the other classes of damage are held constant, and they indicate that heavily frosted and immature kernels have a greater effect on weight per measured bushel than bran frosted or green kernels. It is of interest that, as compared with the simple correlations, the partials involving weight per bushel with bran frost and with green kernels are lower and only barely significant. Green and bran frosted kernels are usually plump and hence should not affect the weight per bushel seriously. The simple correlations are due therefore very largely to the association of these two classes with heavy frost and immaturity.

It is obvious from the nature of the data that the partial correlation $r_{w1.2345}$ would be zero as the sum of 1, 2, 3, 4 and 5 is 100 for all samples except for slight variations due to the very small nondescript class. The multiple $R_{w.2345}$ is analogous to the simple correlation r_{w1} and while of greater magnitude is probably of the same order of significance.

The magnitude of the simple correlation between weight per bushel and percentage of sound kernels, together with the observation that heavily frosted and immature kernels are chiefly responsible for the decrease in weight per bushel, suggests the utility of weight per measured bushel as a grading factor in the commercial grades.

Effect of Storage on Grading

It is believed by some wheat producers that the grain improves in appearance and hence in grade when held in storage over winter. In order to determine whether there was any basis in fact for this belief the entire series of samples which was graded originally in December 1928 were regraded the following July. The changes in grade shown in Table VI in no case exceeded one grade.

TABLE VI

CHANGES IN GRADE DURING STORAGE

Grade in Fall	Number of samples	Samples with changed grade in Spring			
		Higher		Lower	
		Number	%	Number	%
1°	8	0	0.0	2	25.0
2°	44	2	4.5	2	4.5
3°	38	8	21.0	1	2.6
No. 4	51	3	5.7	2	3.8
No. 5	48	7	14.6	1	2.1
No. 6	39	10	25.6	0	0.0
Total	228	30	13.2	8	3.5

The results show that 16.7% of the samples changed in grade, 13.2% receiving a higher grade. The percentage values for the individual grades show that a relatively greater number of samples graded higher in the cases of No. 5 and No. 6. This indicates that there may actually have been a change in physical appearance, particularly in the samples containing a large proportion of damaged kernels. If due allowance is made for borderline samples, the fact that 83.3% of the samples remained unchanged in grade indicates that the consistency of grading is fairly satisfactory for work of this nature where the personal factor plays such an important role.

II. Milling Quality

The commercial value of hard red spring wheat depends upon two factors, the quantity and quality of flour which the wheat is capable of yielding. The first factor, depending upon flour yield, is usually referred to as milling quality, and the latter, which involves flour strength, as baking quality. The classification of wheat into grades, by reference to physical characteristics which may be rapidly determined or estimated, such as weight per bushel and external appearance, is made with the object of classification into groups differing in

wheat quality. The samples in this study were therefore submitted to experimental milling and baking procedures in order to determine the value of such physical characters as are now employed in grading as an index of actual value, and further to ascertain the relative effect of the various forms of damage which occur in increasingly large quantities as the grade decreases.

In experimental milling one of two procedures is usually followed. Either a patent flour, representing some definite percentage of the total flour, or a straight grade which includes all the flour, is milled. When a patent flour is milled the percentage of the total flour it is desired to include is made up from the best portions, and there is some question among cereal chemists as to which is the most desirable procedure from the standpoint of determining the baking quality of a series of wheats.

In this study both procedures were followed, two of the collaborating laboratories milling a 55% patent, and the other essentially a straight grade flour. These two practices were used in order to ascertain whether there would be any marked difference in the relative baking value of the samples when determined by the use of flour of such widely different extractions. It was thought that the relative baking results on these two flours for a series of samples might reveal whether the damage were external or fairly deep-seated. If external, the short patent would be expected to show progressive superiority over the straight grade flour as the percentage of frost damaged kernels increased.

A uniform system of milling was followed in the three laboratories. The wheat samples after cleaning and scouring were conditioned to 13% moisture from four to seven days prior to milling, a 2000-gm. sample was tempered to 15% moisture and milled on an Allis Chalmers experimental mill following the flow sheet published by Geddes (4). To provide for greater uniformity in milling, standard samples of feed flour and shorts were forwarded to each of the collaborating laboratories. Extraction of flour from the shorts was continued until the residue matched the standard and the total flour thus obtained was weighed.

In making up the patent flour, successive fractions obtained by the progressive reduction of the middlings were taken until 55% of the total flour was included. In milling a straight, reduction of the feed flour was continued until the residue matched the standard. In making up the straight, all flour with the exception of residual feed flour was included. The term straight grade flour is hence not strictly correct as in reality a long patent was milled. However, in order to differentiate clearly between the two extractions, the long patent is referred to as a straight grade flour. The mill rooms were maintained at a relative humidity of approximately 70% by means of Bahnson humidifiers. Two of the laboratories were equipped with three-stand experimental mills, and the other, designated as Laboratory A, used a two-stand mill by the same manufacturer. Each laboratory was supplied with 60 lb. of the same lot of 2° wheat, obtained from the Winnipeg Grain Inspection office, which served as a milling standard.

By expressing the total flour yields obtained in each laboratory as a percentage of the mean value for replicate millings of the standard wheat, it was possible to determine whether the different laboratories obtained the same relative results on a particular sample, thus facilitating compilation of the data. Such a procedure was necessary because of considerable differences in the absolute values obtained, the total flour yields reported by Laboratory A being consistently lower. This may have been due in part to the technique of the operator, but more likely to the fact that this laboratory was equipped with a two-stand mill, and probably did not secure as thorough a clean up of the bran. However, in general, the three laboratories obtained the same relative results with the exception that Laboratory A secured relatively lower flour yields for Grade No. 6. For this reason the total flour yields obtained in that laboratory have been recorded separately, in addition to the average values for the three laboratories. In milling according to the method outlined above, it was observed that the yield of straight grade flour did not approximate a constant percentage of the total but progressively decreased as the milling quality of the samples became lower. That is, in milling the lower grade wheats an increasing amount of feed flour which matched the arbitrary standard was obtained. In view of this observation the yield of straight grade flour has been expressed as a percentage of the total flour obtained for Laboratory A. Protein determinations were made on the wheat by the usual Kjeldahl-Gunning procedure and the results expressed on a 13.5% moisture basis.

The mean values for wheat protein and milling results summarized according to wheat grade are given in Table VII, together with the data for weight per measured bushel previously given in Table I. The significances of the trends in the mean values have been determined by analyses of variance and are indicated by the Z values and their respective 5% points at the foot of Table VII. The range of variability within each grade in terms of the standard deviation is also recorded for each set of values.

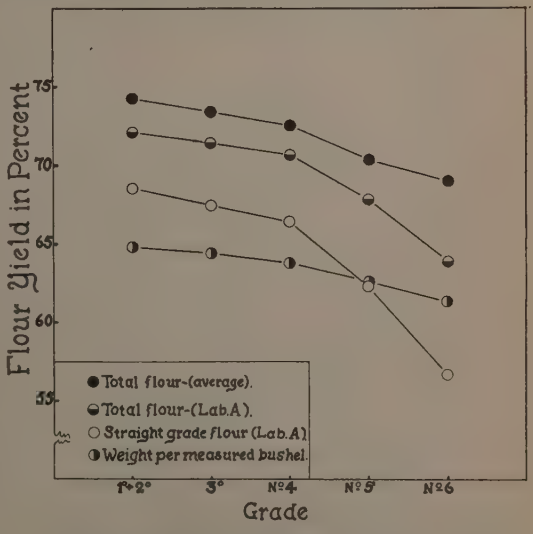


FIG. 2. *Showing the mean yield of total flour, straight grade flour, and mean weight per measured bushel according to wheat grade.*

There is, as might be expected, no significant difference in the mean protein content of the samples in the various grades, but the decrease in the yield of

both total and straight grade flour as the grade is lowered is highly significant. The trends in flour yield and weight per bushel are represented graphically in Fig. 2, and show that Laboratory A, despite a lower flour yield, obtained the same relative results as the three laboratories combined with the exception of the values for grade No. 6.

Before considering the trends in milling quality for the different grades it seemed advisable to determine the extent of the relationship between the three sets of values for flour yield given in Table VII. Simple correlations have accordingly been computed between the percentages of straight flour and the two values for total flour yield. The results by grades and for all grades combined are recorded in Table VIII. It will be noted that there is a very close relationship between the yield of straight grade flour and that of total flour obtained in the same laboratory. The straight grade flour yield is also highly correlated with the average total flour yield of the three laboratories, being however somewhat lower for grade No. 6 as is evident from Fig. 2.

The covariance analysis given in the lower half of Table VIII is useful in showing the relation between the correlated variables, throughout the entire population of samples, for the means of the variables for the corresponding grades, and within the grades. It will be noted that there is a very close relation between the mean, total and straight grade flour yields for the different grades and that there is a very significant relation between these variables

TABLE VII

MEANS, STANDARD DEVIATIONS AND Z VALUES FOR WEIGHT PER MEASURED BUSHEL, PROTEIN CONTENT OF WHEAT, AND FLOUR YIELD

Grade	No. of samples	Weight per measured bushel		Protein in wheat (13.5% M.B.)		Total flour yield. Av. all labs.		Total flour yield Lab. A		Str. grade flour yield Lab. A		Str. grade flour as % total
		Mean lb.	S.D. lb.	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %
1° & 2°	52	64.7	1.3	12.2	1.1	74.0	1.2	72.0	2.0	68.5	2.6	95.1
3°	38	64.3	1.0	11.8	1.2	73.4	1.7	71.4	1.8	67.4	2.0	94.4
No. 4	51	63.7	1.2	12.1	1.2	72.6	1.4	70.6	2.2	66.3	2.3	93.9
No. 5	48	62.5	1.4	12.0	1.3	70.3	1.9	67.6	2.5	62.1	4.1	91.9
No. 6	39	61.3	1.6	12.3	0.9	69.0	2.6	63.8	3.7	56.5	5.0	88.6
Z		1.9309		0.0585		2.0488		2.1662		2.3470		
5% pt.		0.4403		0.4403		0.4403		0.4403		0.4403		

within the grades. The correlation within grades may be considered an estimate of the correlation to be expected within any one grade on the assumption that the actual variations in this value for the different grades are due merely to random sampling. In view of these high and significant correlations, the different measures of milling quality show good agreement and any one set of results for flour yield may be taken as a measure of the relative milling value of the different grades.

TABLE VIII

SIMPLE CORRELATIONS BETWEEN DIFFERENT MEASURES OF MILLING QUALITY

Correlations between	Simple correlation coefficients—wheat grades					
	1° & 2°	3°	No. 4	No. 5	No. 6	All grades
Total flour yield av. all labs. (t_1) and straight flour yield (s). $r_{t_1 s}$	+ .693	+ .625	+ .515	+ .642	+ .433	+ .793
Total flour yield lab. A (t_2) and straight flour yield (s). $r_{t_2 s}$	+ .934	+ .911	+ .855	+ .811	+ .953	+ .960
5% Pt.	.271	.316	.275	.283	.312	.132

Analysis of Covariance for Total Flour Yield (Av.) and Total Flour Yield (Lab. A), with Yield of Straight Grade Flour (Lab. A)

Correlation coefficients for	Straight grade and total flour av.		Straight grade and total flour Lab. A		5% Pt.	Degrees of freedom
	r_{st_1}	t	r_{st_2}	t		
Total, all grades	+ .793	19.5	+ .960	51.9	1.96	226
Between grades	+ .949	5.2	+ .970	6.2	3.18	3
Within grades	+ .586	10.7	+ .807	20.2	1.96	218

The decrease in total and straight grade flour yield is quite uniform to grade No. 4 and then decreases sharply in the lower commercial grades. The falling-off in the yield of straight flour is most marked, the spread between the top and bottom grades being 12.0% as compared with 8.2% for the yield of total flour obtained by the same laboratory. This implies that in commercial practice a greater proportion of the flour from the lower grade wheats would have to be excluded from the top patents in order to obtain flours approximating in color and ash content those obtained from the higher grades. While the spread in flour yield obtained commercially between the top and bottom grades probably differs somewhat in magnitude from that obtained experimentally, because of the more refined machinery employed, the experimental milling results show clearly that the grading system gives on the average a satisfactory differentiation of wheat on the basis of milling quality.

It should also be mentioned that considerable difficulty was experienced in the reduction of the middlings in the instance of low grade wheats. Owing to the tough and fibrous nature of the middlings, there was approximately a 20% increase in the time required to mill a sample of No. 5 and No. 6 wheat as compared with the statutory grades. This implies that in commercial practice the output of the mill would be lowered and the overhead cost per barrel of flour increased when low grade wheats are milled.

Aside from milling yield, the color and ash content of the flour are important factors in its commercial valuation. At the present time color of flour cannot

readily be evaluated quantitatively, and hence in this study no effort has been made to investigate this factor. It was noted, however, that, in general, the flours from the lower grades of wheat were distinctly inferior in color to those of the top grades. The wheats in grades No. 5 and No. 6 for the most part yielded flours possessing a "dull" or grey color, while the flours from the higher grades were "creamy" or "creamy yellow".

The ash content of several samples of straight flour was determined by the "straight ash" method (A.O.A.C. method) of the American Association of Cereal Chemists (1). The mean results expressed on a 13.5% moisture basis are given in Table IX. The Z value exceeds the 5% point and hence the differences in the mean values are significant. This is due to the higher ash content of flours milled from grade No. 5 and No. 6 wheats as the means for the other grades are identical. The range is only 0.03% and would probably have been greater had the straight grade flour represented a constant percentage of the total for the different grades. The data indicate, however, that it would be necessary to include still less of the total flour in the straight, in the instance of low grade wheats, in order to approximate the ash content of the flours milled from the higher grades. The difference in the commercial value of the various grades from the standpoint of milling quality may hence actually be greater than that indicated by the ranges in flour yield obtained experimentally by the methods employed in this study.

TABLE IX

MEAN ASH CONTENT OF STRAIGHT GRADE FLOURS

Grade	Number of samples analyzed	% Ash (13.5% m.b.)
1° and 2°	21	0.46
3°	13	0.46
No. 4	21	0.46
No. 5	30	0.49
No. 6	20	0.49
Z		0.8248
5% Point		0.4507

While a study of the mean flour yields of the several grades reveals that they are significantly different in respect to milling quality, the variability in flour yield within a grade is also of importance, not only to the miller, but particularly to the wheat producer who is interested in the grading of his own relatively small parcel of wheat.

The standard deviations given in Table VII show that the general tendency is for variability to increase in the lower grades and this increase is especially marked for grade No. 6. A suitable test for the trend in the standard deviations from the higher to the lower grades is somewhat difficult to devise as the grade numbers do not represent numerical values, but it is possible to test any one standard deviation against another or to arrange them in groups and test one group against another. A logical method is to place all the contract grades in one group and all of the commercial grades in another. This has been done for the variability in total flour yield obtained by Laboratory A and also for the three laboratories combined. The results of the test are given in Table X in terms of Z values and their 5% points. The Z values are quite significant, showing that the variability in flour yield is appreciably higher in the commercial than in the contract grades.

Relation between Physical Characteristics of Wheat and Milling Quality

The relation between test weight per bushel and flour yield is shown by the simple correlation coefficients given in Table XI. The correlations between total flour yield, straight flour yield, and weight per measured bushel for the entire series are very significant and their magnitude reveals, in agreement with numerous reports in the literature, that test weight per bushel is a valuable index of flour yield. The correlations for individual grades are much lower and in some instances are not significant. Hence the magnitude of the total correlation is due largely to the differences between the grades. In other words the correlation between the mean flour yields and mean weight per bushel for the five grades must be high. This is obvious from Fig. 2.

TABLE X

COMPARISON OF VARIABILITY IN TOTAL FLOUR YIELD IN THE CONTRACT AND COMMERCIAL GRADES

Grades	Sums of squares total flour yield		Degrees of freedom	Variance	1/2 log e	Z	5% Pt.
All laboratories							
1° & 2°	81.0	192.0	88	2.182	0.3900		
3°	111.0						
No. 4	101.0					0.3047	0.1632
No. 5	177.6	541.6	135	4.012	0.6947		
No. 6	263.0						
Laboratory A							
1° & 2°	203.8	333.7	88	3.791	0.6664		
3°	129.9						
No. 4	248.8					0.4388	0.1632
No. 5	311.1	1105.7	135	8.193	1.1052		
No. 6	545.8						

Since weight per bushel is not an important factor in grading wheat into the commercial grades Nos. 4, 5, and 6, it is of interest to examine the correlations between weight per bushel and flour yields within the grades. For the average total flour yield the correlation falls off slightly from grades 1° and 2° to No. 6, but for yield of straight grade flour there is no evidence of such a trend. Also, in referring to Fig. 2 it will be observed that the drop in weight per bushel from No. 4 to No. 6 is even greater than from grades 1° and 2° to No. 4. Consequently, from the standpoint of flour yield, weight per bushel is just as useful a factor in determining the lower grades as it is in determining the higher grades.

TABLE XI

SIMPLE CORRELATIONS BETWEEN WEIGHT PER MEASURED BUSHEL AND FLOUR YIELD

Correlations between	Simple correlation coefficients. Wheat grades					
	1° & 2°	3°	No. 4	No. 5	No. 6	All grades
Weight per bu. (w) and total flour yield. Av. all labs. (t); r_{wt}	+ .507	+ .279	+ .146	+ .266	+ .338	+ .641
Weight per bu. (w) and straight flour yield. Lab. A (s); r_{ws}	+ .398	+ .302	+ .503	+ .284	+.566	+ .723
5% Pt.	.271	.316	.275	.283	.312	.132

The simple, fourth order partial and multiple correlations for all grades combined, given in Table XII, summarize the relation between flour yield, weight per bushel and classes of kernels. It is obvious from this table that flour yield is closely and positively correlated with weight per bushel and also with percentage of sound kernels, and that these physical characteristics are practically equal in value as a basis for the classification of wheat samples with respect to flour yield.

TABLE XII

SIMPLE, FOURTH ORDER PARTIAL AND MULTIPLE CORRELATIONS BETWEEN FLOUR YIELD AND PHYSICAL CHARACTERISTICS OF WHEAT

	Simple correlations		Fourth order partial correlations			
	Total flour yield av. all labs. (t)	Straight grade flour yield (s)	Total flour yield average all labs. (t)		Straight grade flour (s)	
Total sound (1)	+ .716	+ .717				
Bran frost (2)	− .459	− .515	$r_{t2.345w}$	− .318	$r_{s2.345w}$	− .388
Heavy frost (3)	− .561	− .546	$r_{t3.245w}$	− .467	$r_{s3.245w}$	− .388
Immature (4)	− .395	− .349	$r_{t4.235w}$	− .408	$r_{s4.235w}$	− .293
Green (5)	− .312	− .384	$r_{t5.234w}$	− .074	$r_{s5.234w}$	− .172
Weight per bushel(w)	+ .641	+ .722	$r_{tw\ 2345}$	+ .337	$r_{sw\ 2345}$	+ .507
	At 5% Pt. = .132		At 5% Pt. r = .133			

MULTIPLE CORRELATIONS		R	5% point
$R_{t(2345w)}$	=	.794	.322
$R_{s(2345w)}$	=	.832	.322

The partial correlations of the fourth order measure the relationship between milling quality, test weight per bushel, and forms of damage, when each of the factors varies alone. It will be noted that bran frost, heavy frost, and immature kernels are negatively correlated with flour yield and are of approximately equal importance in their effects. As would perhaps be expected, the green kernels have very little effect on flour yield when the other variables are held constant. The multiple correlations are quite high and show that the various forms of damage combined with test weight per bushel give a very good indication of flour yield.

Since the forms of damage (2, 3, 4, and 5) are the complement of "total sound", and the effects of each class of damage when varied alone are practically equal and of a low order, it is evident that the percentage of sound kernels without regard to the particular forms of damage present, together with weight per measured bushel, gives the best indication of the probable milling value. While the data suggest that green kernels have somewhat less influence on flour yield than the other classes of damaged kernels, they normally constitute, as may be seen from Table I, such a small proportion of the total damage that no appreciable error would be expected by including them with the other forms of damage without special consideration.

III Baking Quality

"Baking quality" is used here synonymously with flour strength as a term to designate in a general way those characteristics of a flour which make it desirable for bread making purposes. The experimental determination of the quality of Canadian wheat in so far as flour strength is concerned presents a difficult problem. A particular parcel of wheat under investigation is almost invariably mixed for domestic consumption with other lots of hard red spring wheat, or blended with a wide variety of softer wheats if sold on the foreign market. Furthermore, the resulting flours are baked under widely varying conditions with regard to baking formulas, methods of mixing and fermentation time. Finally the character of the loaf desired differs according to country and locality.

The problem of evaluating the baking quality of Canadian wheat is simplified, however, by the fact that sound Marquis wheat from the southern portions of the prairie provinces is universally recognized as the standard of quality, and such wheat thus provides a standard on which to base comparisons. Until recently the baking formula in most common use in cereal laboratories included only flour, water, yeast, sugar and salt as ingredients, but Larmour and MacLeod (8, 9) have adduced abundant evidence that such a formula often gives incongruous results when applied to flours experimentally milled from Western Canadian wheat. The addition of potassium bromate to the formula, or a blend of the flour under study with a weak flour, however, gave results much more consistent with the commercial evaluation of Canadian wheats.

In view of these considerations it has been deemed essential, in the collaborative work of the Associate Committee on Grain Research, to apply different baking formulas which are designed to reveal the baking characteristics of the flours under several representative conditions. The formulas used in the present study were as follows:

1. Unblended Flour

(a) "Simple" formula—including only flour, water, yeast, sugar and salt.
(b) "Bromate" formula—Simple formula with the addition of 0.001 gm. potassium bromate, an improver which affects gas retention.
(c) "Malt" formula—Simple formula with the addition of 1.0 gm. Fleischmann's diastatic malt, which affects gas production.

2. Blended Flour

"Blend" formula—the flour under investigation was blended with 10% corn starch and baked by the bromate formula.

In the blend formula corn starch was used since it has been found to give results essentially similar to the addition of 40% of a weak flour and has the advantage of being more uniform in composition. The blend was baked by the bromate formula to compensate for the limited aging which experimentally milled flours receive before being submitted to the baking test.

Aside from variations in the baking formulas the baking procedure is standardized and is similar, with certain minor modifications, to the tentative "Basic Standard Procedure" of the American Association of Cereal Chemists as outlined by Blish (2). The basic procedure is followed with respect to the proportions of yeast, salt, and sugar, with the difference that 100 gm. of flour on a 13.5% moisture basis (determined by the vacuum oven) is used instead of a 15.0% moisture basis. The absorption is varied in order to produce a dough of the desired consistency, the doughs being mixed in a small Hobart mixer, equipped with two hooks, operated at No. 2 speed for three minutes. The loaves are baked in pans with low sides (top, $4\frac{1}{4}$ by $2\frac{3}{4}$ in.; bottom, $3\frac{11}{16}$ by $2\frac{1}{6}$ in.; depth, 2 in.). In all other respects the conditions laid down in the basic procedure of the A.A.C.C. are followed. Loaf volume is determined in a measuring device similar to that described by Geddes and Binnington (5) or in that described by Malloch and Cook (11). In all tests duplicate loaves were baked on different days, and if the duplicates failed to check within 20 cc. the test was repeated. Larmour, Machon, and Brockington (10) have fully described the baking routine in the laboratories collaborating in the work of the Grain Research Committee and further details may be obtained from their paper.

The loaves were judged the day following baking, the characteristics of the bread being scored on a numerical scale. Where a score less than perfect is assigned, a word or letter is appended indicating the type of fault; where no letter is given the fault is not well defined. The following factors were considered in scoring the loaves, and in assigning the key letter.

A. General Appearance

(1) Crust color — Perfect score 5.
p = color paler than ideal; d = color darker than ideal.

(2) Form — Perfect score 5.
Under this heading are included external characteristics other than crust color.
g = green, or underfermented characteristics; o = overfermented characteristics; f = flat, loaf not well risen; s = shell top.

B. Crumb Character

(1) Grain and texture — Perfect score 10.
Grain and texture are considered together, the usual significance of these terms being employed.

c = coarse — thick cell walls; o = open — large cells; close = small, round cells;

(2) Crumb color — Perfect score 10.
d = dull; g = grey; y = yellow.

In order to secure a single figure expressive of bread quality an empirical baking score is computed by summing the scores for the bread characteristics weighted in the following manner:

(Loaf volume − 400) × 0.2	=
Crust color + form	=
Crumb texture × 3	=
Crumb color × 2	=
(Absorption − 60)	=
Baking score	———

While the evaluation of characteristics other than volume is subject to personal judgment, the scoring in the collaborating laboratories has been made reasonably uniform by the use of reference standards and by occasional conferences where loaves differing widely in general appearance, crumb color, and texture are judged by the different operators. The work in the three laboratories is further standardized by the inclusion of a reference flour in each day's bake, the judging scores assigned to this standard being decided in advance of the baking of the experimental series. Despite a concerted attempt at standardization, it is not surprising that differences in loaf volume are obtained on the same sample in the different laboratories. The relative mean volumes for the standard flour in each laboratory, however, serve as a basis for summarizing the data. By expressing the absolute volume obtained in each laboratory as a percentage of their respective mean value for the standard, the extent of agreement can be ascertained and these percentage values averaged. The resulting figures may then

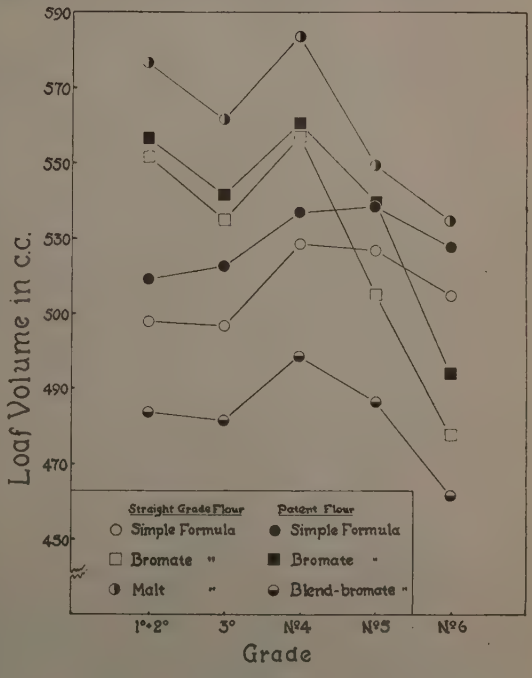


FIG. 3. *Showing the mean loaf volumes for the various grades by different baking formulas.*

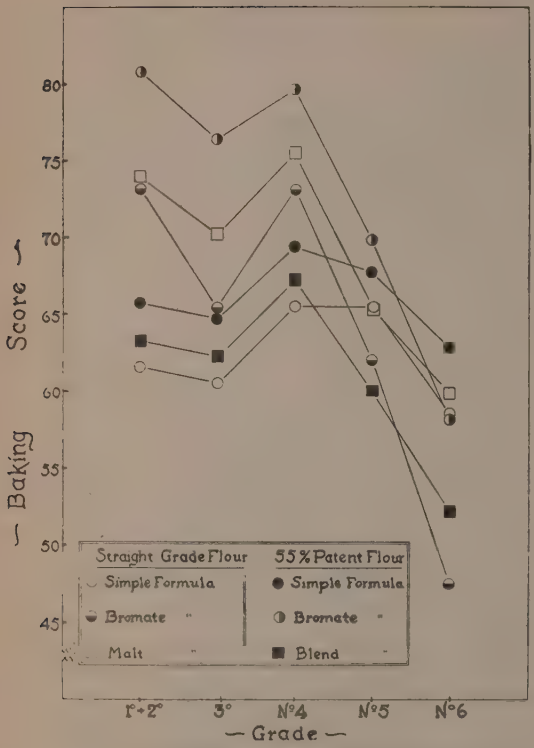


FIG. 4. *Showing the mean baking scores for the various grades by different baking formulas.*

be reconverted to loaf volume by utilizing the combined mean value obtained for the standard flour in the three laboratories, thus reducing all the results to a comparable basis.

In this study, the flours were allowed to age approximately one month before baking. The 55% patent flour was baked by the simple, bromate and blend formulas and the straight grade flour by the simple, bromate and malt formulas. The baking tests on the straight flour were all conducted in one laboratory, the patent flour was baked by the simple formula in two laboratories, while the bromate and blend formulas were applied to the patent flour in different laboratories.

The means, standard deviations and Z values for the baking characteristics obtained on the patent and straight grade flours are summarized in Table XIII.

The Z values given at the foot of each section of the table with their respective 5% points show the significance of the differences in the mean values recorded in the same column. It will be noted that the mean loaf volumes and computed baking scores recorded for the various grades are all significantly different with the exception of those obtained by the simple formula. The Z values for loaf volume obtained by this method on the straight flour however just exceeds the 5% point.

The mean loaf volumes and baking scores for both the patent and straight grade flours using the different baking formulas are represented graphically in Figs. 3 and 4 respectively, and illustrate the nature of the trends in these values for the various grades.

It will be observed from Fig. 3 that the baking quality as measured by loaf volume shows an increase for the simple formula from grades 1° and 2° to grade No. 5 then falls off in grade No. 6. Since the simple formula failed to reveal any significant difference in the mean loaf volumes in the instance of the patent flour and just exceeded the level of significance in the case of the straight flour, little importance can be attached to the trend indicated in the graph. If regard is paid to the trend, it reveals a progressive improvement in baking quality as measured by loaf volume as the grade of wheat decreases to No. 5.

TABLE XIII

MEANS, STANDARD DEVIATIONS AND Z VALUES FOR THE BAKING CHARACTERISTICS OBTAINED BY DIFFERENT BAKING FORMULAS

Grade	No. of samples	Absorption (13.5% M.B.)		General appearance		Crumb color		Crumb texture		Loaf volume		Baking score	
		Mean %	S.D. %	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean cc.	S.D. cc.	Mean	S.D.
Simple Formula—Straight Grade Flour													
1° and 2°	52	64.0	1.68	7.7	0.58	6.1	1.08	5.4	0.94	507.7	33.22	61.6	10.31
3°	37	64.7	2.01	8.0	0.79	5.5	0.92	5.1	1.22	506.5	33.00	60.7	10.73
No. 4	50	65.0	1.54	8.0	0.74	5.7	1.33	5.4	1.21	528.5	41.24	65.8	13.12
No. 5	47	67.0	1.86	8.0	0.96	5.1	1.56	5.0	1.56	526.8	52.75	65.8	17.46
No. 6	36	68.3	1.70	7.5	1.21	4.0	1.16	4.4	1.49	514.6	56.65	58.7	16.88
Z		1.8903		0.5191		1.3953		0.6911		0.4641		0.3853	
5% Pt.		0.4404		0.4404		0.4404		0.4404		0.4404		0.4404	
Simple Formula—55% Patent Flour													
1° and 2°	52	64.5	0.99	7.1	0.86	6.4	1.10	5.9	1.26	519.0	28.11	65.9	9.25
3°	38	65.1	1.55	7.1	1.18	5.9	1.02	5.4	1.20	522.6	29.61	64.9	9.35
No. 4	51	65.1	1.29	7.6	1.05	6.2	1.09	5.7	1.18	536.6	37.00	69.5	10.42
No. 5	48	66.1	1.74	8.0	1.19	5.2	1.59	5.1	1.71	538.5	55.32	68.0	16.65
No. 6	39	67.9	2.10	8.2	1.26	4.1	1.58	4.4	1.66	527.7	58.81	63.3	18.08
Z		1.7219		1.0836		1.5514		0.9780		0.3055		0.2255	
5% pt.		0.4403		0.4403		0.4403		0.4403		0.4403		0.4403	
Bromate Formula—Straight Grade Flour													
1° and 2°	50	63.9	1.62	8.0	0.89	6.3	1.00	6.1	1.26	551.9	56.24	73.2	16.17
3°	36	64.6	2.04	7.9	1.04	5.7	1.12	5.7	1.41	534.2	59.50	65.5	17.23
No. 4	49	65.0	1.60	7.9	0.98	6.1	1.12	5.7	1.25	556.7	63.09	73.1	17.29
No. 5	43	66.8	1.77	7.2	1.17	5.0	1.31	4.4	1.18	515.8	76.67	62.2	19.12
No. 6	33	68.1	1.66	6.5	1.05	3.6	1.06	3.7	1.21	477.3	70.95	47.3	18.79
Z		1.8093		1.3125		1.7732		1.6152		1.1624		1.2976	
5% pt.		0.4408		0.4408		0.4408		0.4408		0.4408		0.4408	
Bromate Formula—55% Patent Flour													
1° and 2°	48	65.0	1.65	7.1	1.37	7.2	1.95	8.0	1.36	556.4	59.56	80.9	18.18
3°	34	65.1	2.09	7.3	1.58	6.7	1.75	7.1	2.36	541.6	62.33	76.3	22.47
No. 4	43	64.9	1.81	7.3	1.69	7.2	1.67	7.6	1.76	560.2	65.09	79.7	20.61
No. 5	39	66.0	1.84	7.2	1.91	5.3	2.31	6.1	2.46	539.6	76.28	69.8	25.02
No. 6	34	68.1	2.38	6.5	2.01	3.9	2.35	4.9	2.49	494.0	70.92	57.8	22.46
Z		1.4237		0.1669		1.4608		1.2974		0.9277		1.5485	
5% pt.		0.4414		0.4414		0.4414		0.4414		0.4414		0.4414	
Blend Formula—55% Patent Flour													
1° and 2°	52	65.0	1.05	8.4	0.94	6.7	1.01	6.4	1.00	483.7	33.18	63.3	10.29
3°	38	65.4	1.11	8.5	1.09	6.5	1.02	6.4	1.16	481.6	36.88	62.1	12.20
No. 4	51	65.4	0.91	8.7	0.79	6.7	1.02	6.5	0.98	498.7	36.17	67.2	11.79
No. 5	48	67.1	2.52	8.6	1.26	5.4	1.47	5.4	1.49	486.4	55.10	60.0	16.71
No. 6	39	69.4	2.36	8.2	1.39	4.2	1.42	4.6	1.60	461.7	52.13	52.2	16.96
Z		1.9322		0.1578		1.7914		1.4223		0.6967		0.9534	
5% Pt.		0.4403		0.4403		0.4403		0.4403		0.4403		0.4403	
Malt Formula—Straight Grade Flour													
1° and 2°	52	62.9	1.60	7.8	0.50	5.9	1.03	5.3	1.22	576.7	47.63	73.8	12.37
3°	38	63.7	2.07	7.7	0.76	5.6	1.16	5.2	1.24	561.5	42.46	70.3	13.37
No. 4	51	64.0	1.70	7.7	0.79	5.8	1.35	5.2	1.13	583.3	45.03	75.8	13.34
No. 5	48	66.0	1.88	7.2	1.16	4.6	1.31	4.2	1.27	549.1	68.64	65.5	19.41
No. 6	39	67.4	1.61	6.6	1.05	4.0	1.34	3.8	1.20	534.5	66.74	60.1	18.33
Z		1.9305		1.3002		1.4814		1.2962		0.8280		0.9968	
5% Pt.		0.4403		0.4403		0.4403		0.4403		0.4403		0.4403	

This result is entirely incongruous with the commercial evaluation of the grades and confirms the observations previously published by Larmour and MacLeod (9) in regard to the value of the simple formula. It should be added that the data for the straight grade flour obtained in one laboratory are in close relative agreement with the average results of the two laboratories which tested the patent flour.

It should be pointed out here that the simple formula as applied to experimentally milled flours is not comparable to commercial practice. Experimental flours are unbleached and usually receive little or no aging before being submitted to the baking test. Commercial flours are normally aged for a much longer period, and frequently bleached with agents which act also as flour improvers, and in addition may have an improver added in the commercial bakeshop.

The trends in loaf volume by all the other baking formulas are highly significant and are quite similar. In each instance the curve falls from 1° and 2° to 3°, rises sharply to a maximum value in grade No. 4 and then declines abruptly for grades No. 5 and No. 6. It is of interest to note the close parallelism in the two curves for the results by the bromate formula. The same general trends are evident for the baking scores as will be seen from the graph.

Considering now the trends in the means for the individual baking characteristics, absorption, general appearance, crumb color, and crumb texture given in Table XIII, these are all significant with the exception of general appearance as recorded for loaves baked by the bromate and blend formulas.

Absorption increases markedly from grades 1° and 2° to grade No. 6 for all the baking formulas, the absolute values being somewhat lower for the malt formula. The doughs containing malt were slightly sticky and showed a tendency to "slacken" during fermentation, and for this reason less water was used in mixing. High absorption is a characteristic of flours milled from frosted wheat and in individual cases ran as high as 75%.

The means for general appearance, crumb color, and crumb texture, show, with the exception of No. 4, a fairly progressive trend towards lower values as the grade decreases. As the type of fault for which the loaves were scored down varied considerably within each grade it was not possible to affix a key letter describing the various loaf characteristics in the table of means.

In a general way, the external appearance of the loaves was quite similar until grade No. 6 was reached when the crust color possessed a characteristic dull hue. The crumb texture was quite similar for grades 1° and 2° to No. 4, that of grade No. 4 being equal on the average or superior to the scores recorded for 3°. The textures of the loaves baked from flours milled from No. 5 and No. 6 wheats however were distinctly inferior, being "open" and possessing thick cell walls. The crumb colors recorded for grade No. 4 were superior to those for 3°, varying from "creamy" to "creamy yellow". The predominating crumb color in the case of grade No. 5 was a "dull creamy" and in grade No. 6, "dull grey".

The grade No. 4 wheats thus yielded, on the average, flours which possessed superior baking quality to 3° as measured not only by loaf volume, but also

by crumb color and crumb texture. Although the present grading system gives a satisfactory differentiation of baking quality when the contract grades are considered as one group and the commercial grades as another, the transition from the one to the other, that is from 3° to No. 4, is not satisfactory. It is, therefore, not surprising that, in the instance of the 1928 crop, there was a frequent preference among British millers for "fours" rather than "threes" on the basis of quality independent of price. Since, in the 1930 Amendments to the Canada Grain Act, the definition of 3° was changed to require it to contain 25% hard vitreous kernels this anomaly is not likely to recur.

A reasonable explanation suggests itself for the anomalous relationship between 3° and No. 4. Samples which are graded into the contract grades are composed principally of sound kernels, the distinction between the grades being made chiefly on the basis of percentage of starchy kernels. Three Northern, therefore, represents the poorest portion of the sound samples. In a frosted wheat crop, such as that of 1928, the differentiation of the commercial grades is made on the basis of percentage of visible damage, No. 4 being, of course, the least damaged of the group. Had they not been frozen, it may be assumed that the samples grading No. 4 would have been divided among the contract grades. Thus, in effect, the average quality of the samples with the highest percentages of starchy kernels is being compared with the average quality of samples ranging from entirely vitreous to entirely starchy but damaged in appearance by frost and the presence of immature and green kernels. Although the latter wheats were damaged in appearance, these samples in reality had a higher baking quality, and it must be concluded that the actual damage due to the presence of frosted, immature and green kernels was not sufficient to mask the inherent higher quality of the samples in grade No. 4. The foregoing explanation does not completely explain the results since the grade No. 4 wheats showed superior strength not only to those of 3°, but also to the averages of 1° and 2°. It must be recalled, however, that only nine samples grading 1° were included in this study. If a sufficient number of representative samples for this grade had been collected, it would have been possible to consider the results for 1° separately, and would serve as a basis for indicating the probable correctness of the suggested explanation. The further possibility of improvement in the baking quality of No. 4 wheat as a direct result of the presence of a limited number of frosted kernels is now under investigation.

Relation Between Loaf Volumes Determined by Different Baking Formulas

In order to measure the extent of the relationship between the loaf volumes obtained by the various baking formulas employed, the simple correlations given in Table XIV have been calculated. The first two sets of correlations given in the table are of a high order and indicate that the straight grade and patent flours give the same relative results when comparisons are made by either the simple or by the bromate formulas.

Correlations within grades were determined by Z transformation (3) and were approximately the same as for the entire population. Correlations between the mean values for the different grades were calculated for r_{ac} and r_{eg} and

were + .952 and + .907 respectively. These correlations show that all of the samples may be looked upon as a uniform population, about the same relation existing between as within the grades.

TABLE XIV

THE RELATION BETWEEN LOAF VOLUMES OBTAINED BY DIFFERENT BAKING FORMULAS

	r	Simple correlation coefficients—wheat grade						
		1° and 2°	3°	No. 4	No. 5	No. 6	Within grades	All grades
Simple formula straight flour (*a*)× simple formula patent flour (*c*)	r_{ac}	+0.754	+0.821	+0.672	+0.842	+0.967	+0.839	+0.839
Bromate formula straight flour (*e*)× bromate formula patent flour (*g*)	r_{eg}	+ .681	+ .818	+ .851	+ .884	+ .821	+ .819	+ .837
Simple formula straight flour (*a*)× blend formula patent flour (*b*)	r_{ab}	+ .429	+ .750	+ .632	+ .805	+ .771	+ .686	+ .696
Simple formula patent flour (*c*)× blend formula patent flour (*b*)	r_{cb}	+ .574	+ .669	+ .755	+ .818	+ .893	+ .758	+ .762
Bromate formula straight flour (*e*)× blend formula patent flour (*b*)	r_{eb}	+ .665	+ .783	+ .774	+ .884	+ .829	+ .795	+ .798
Bromate formula patent flour (*g*)× blend formula patent flour (*b*)	r_{gb}	+ .580	+ .773	+ .695	+ .869	+ .882	+ .774	+ .785
Approximate value of r at 5% pt.		.27	.32	.28	.28	.31	.13	.13

In this study, baking tests were conducted on both a long patent (here called straight) and a short patent in view of the possibility that they would not give the same relative results on wheats containing a high percentage of frosted kernels if the frost damage were chiefly external in character. The correlations suggest that in experimental determinations of wheat strength it makes little difference whether a patent or a straight flour is milled. This result is of considerable importance in a cereal laboratory as the milling of a straight yields more flour from a given quantity of wheat, thus permitting a more thorough investigation of the baking behavior.

The results by the bromate formulas are highly correlated with those by the blend, while the correlations between the data for the simple and blend are not of such a high order of magnitude. In the latter case in particular, there is some indication of a trend in the correlations within grades towards higher values as the grade decreases.

Response to Differential Baking Tests

Information of practical and scientific interest may be gained by a study of the differences between the results obtained by the different baking formulas. Table XV shows the mean responses in loaf volume and baking scores to potassium bromate, to malt and to blending, using the results by the simple formula with the same kind of flour as a base. The significant Z values show that the trends toward lower mean responses for each of the differential baking tests as the grade of wheat decreases are highly significant. The graph

presented in Fig. 5 showing the mean responses in loaf volume illustrates the nature of these trends more clearly. The curves for the two bromate responses parallel each other and indicate the close relative agreement not only between the two laboratories conducting the tests, but also in the information given by the patent and straight grade flours. The mean response to bromate and to malt of the flours milled from No. 4 wheats is of the same order of magnitude as for 3°.

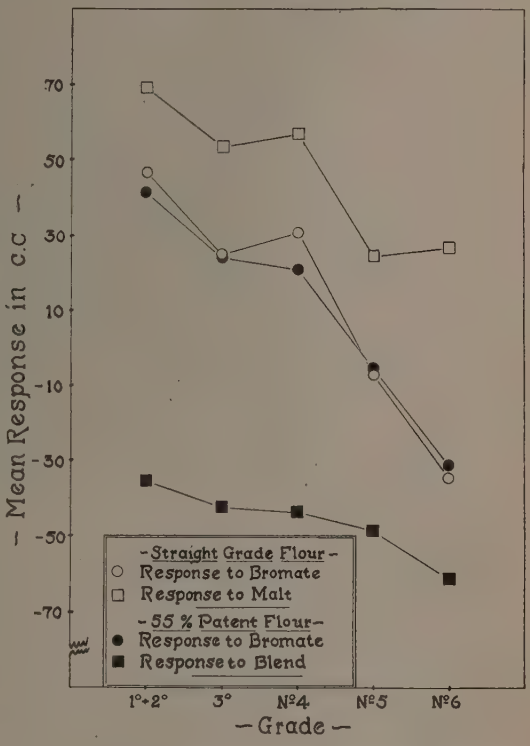


FIG. 5. *Showing the mean responses in loaf volume to differential baking tests.*

The literature dealing with the bromate differential test has been reviewed by Larmour and MacLeod (8) and need not be detailed here. The precise action of potassium bromate is not known but it influences gas retention by some effect, direct or indirect, on the protein, but has no measurable influence on gas production, and the magnitude of the response is associated in part with protein quantity and in part with other factors usually designated as protein "quality", as yet little understood. Flours giving a strong positive response to the bromate differential test are regarded commercially as possessing reserve strength and a high fermentation tolerance. On the other hand, flours showing no appreciable response usually show high baking quality by the simple formula and have little or no reserve strength, while a negative response to bromate indicates weakness and a lack of ability to produce good bread unless handled under ideal conditions.

A high response to malt indicates that the gas production is not sufficient to make full use of the gas-retaining capacity of the dough, while a low response indicates that the gas production is already adequate or that gas retention is poor. The response to the blend formula is obviously a measure of the reserve strength or blending capacity of the flours and is especially important in the testing of quality of Western Canadian wheat which is largely sold for export for blending purposes.

TABLE XV

MEAN RESPONSES IN LOAF VOLUME AND BAKING SCORE TO FLOUR IMPROVERS OR BLENDING, STANDARD DEVIATIONS AND Z VALUES

Grade	Loaf volume in cc.								Baking score							
	Response to bromate				Response malt		Response blend		Response to bromate				Response malt		Response blend	
	Straight grade flour		55% patent flour		Straight grade flour		55% patent flour		Straight grade flour		55% patent flour		Straight grade flour		55% patent flour	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1° and 2°	46.3	45.96	41.1	56.92	69.0	32.88	−35.6	29.68	11.9	15.62	16.6	16.11	12.4	8.67	−1.7	9.12
3°	24.8	50.64	24.0	52.86	53 6	33.42	−42.5	28.80	3.7	15.50	14.6	17.71	9.9	8.62	−3.3	8.29
No. 4	30.2	52.92	.20 6	46.38	56.4	34.26	−43.9	33.42	8.0	15.44	11.4	15.19	9.6	9.03	−3.1	11.39
No. 5	− 7.3	48.60	− 5.2	40.86	24.1	39.06	−48.6	33.60	− 5.1	15.11	1.5	13.03	0.1	9.44	−6.3	11.22
No. 6	−34.3	39.48	−31.4	45.30	26.2	36.12	−61.4	37.98	−11.6	12.76	− 5.6	13.04	1.3	8.44	−10.9	11.39
Z	1.4157		1.2669		1.3123		0.6382		1.3456		1.3307		1.4274		0.8217	
1% pt.	.6139		.6146		.6130		.6186		.6139		.6146		.6130		.6126	

On the basis of the above discussion the significant decreases in the mean responses to the differential tests from grade to grade represent inherent differences in baking behavior. Flours milled from the lower grade wheats, aside from their inferior baking quality, must be regarded as being less desirable commercially because of lower blending strength and the indications of poor fermentation tolerance. In general, the grading system satisfactorily differentiates wheats in these respects although No. 4 is somewhat out of line, giving responses to malt and bromate of the same order as 3°. The standard deviations given in Table XV, however, show that the variability in the responses within each grade is very high.

Since the mean protein content is practically the same for the various grades, the differences in mean response must be attributed largely to differences in protein quality, and it is of practical and scientific interest to evaluate the relative importance of quantity and quality. For this purpose simple, partial, and multiple correlations for some of the responses have been calculated for all grades combined. The results are given in Table XVI. The simple correlations between wheat protein and response to bromate, malt, and blending, indicate that the magnitude of the responses are in part associated with the quality of protein present in the flour.

Taking the response to bromate as representative, the second order partial correlations and the multiple given in Table XVII have been calculated in order to determine the relation betwen protein content, percentage of sound kernels and bromate response. The correlation $r_{6B.12}$ gives the relation between per cent wheat protein and bromate response when the percentage of total sound and bran frosted kernels are held constant. The correlation $r_{1B.62}$ shows the relation between bromate response and percentage sound kernels with protein and bran frost constant. Since "heavy damage" (the sum of heavily frosted, immature and green kernels) designated by key figure 7 is the complement of total sound and bran frost, it is obvious that $r_{7B.62}$ = approximately $-.407$.

TABLE XVI

CORRELATIONS INVOLVING RESPONSES TO DIFFERENTIAL BAKING TESTS

	Simple correlation coefficients					Second order partial correlation coefficients for response to bromate (str. flour)
	Wheat protein (6)	Total sound (1)	Bromate response (str. flour) (B)	Malt response	Blend response	
Wheat protein (6)			+ .319	+ .316	+ .148	$r_{6B.12}$ = + .469
Total sound (1)	− .031		+ .527			$r_{1B.62}$ = + .407
Bran frost (2)	+ .183	− .785	− .344			
At 5% pt. r = .138						At 5% pt. r = .139

MULTIPLE CORRELATION: $R_{B(62)}$ = .625; 5% pt. = .240

It seems logical to presume that the inherent protein quality of frosted, green and immature kernels would be inferior to that of sound kernels and if this assumption is made, the data show that bromate response is associated, in part at least, with the quantity and quality of the protein. The multiple showing the combined effect of protein content and percentage sound kernels is not sufficiently high to suggest that these are the only factors involved in the response to bromate.

TABLE XVII

REGRESSION OF VARIABILITY IN BAKING QUALITY ON MEAN PERCENTAGE OF TOTAL SOUND KERNELS IN EACH GRADE

Grade	Mean total sound kernels %	Variability of		
		Loaf volume bromate formula straight flour σ_e	Loaf volume blend formula patent flour σ_b	Baking score blend formula patent flour σ_L
1° and 2°	85.5	56.24	33.18	10.29
3°	74.2	59.50	36.88	12.20
No. 4	58.6	63.09	36.17	11.79
No. 5	30.8	76.67	55.10	16.71
No. 6	18.6	70.95	52.13	16.96
Regression coefficient	b	−0.285	−0.339	−0.105
	t	4.35	4.35	6.03
	5% Pt.	3.18	3.18	3.18

Variability in Baking Quality Within Grades

The variability of the baking characteristics within each grade is recorded in Table XIII in terms of the standard deviation. Taking loaf volume and baking score as representative of baking quality it will be noted that there is a trend towards increased variability in the lower grades. The significance of this regression of variability on grade has been determined using the variability in loaf volume and baking score by the blend formula, and loaf volume by the bromate formula (straight flour) as typical examples. In order to determine the significance of the regression, the mean percentage of sound kernels has been taken as the best available measure of grade since the grade numbers themselves do not represent numerical values. The regression coefficients are given in Table XVII with their respective t values and 5% points. The trend towards higher variability in the baking quality of the lower grades is highly significant. It will be observed, however, that the variability in grade No. 4 is of the same order as that in the contract grades.

The Relation Between Wheat Protein and Baking Quality

The relation between wheat protein and baking quality as measured by loaf volume for each baking formula is given in Table XVIII for the individual grades and for all grades combined. The correlations are not of a high order,

those for the bromate, blend and malt formulas being of greater magnitude than the corresponding values for the simple formula. The significance of the difference in the correlations by the various baking methods has been determined for the entire series. This is expressed by the ratio of the difference in Z values for each pair of correlation coefficients $(Z_1 - Z_2)$ to its standard deviation $\sigma_{(Z_1-Z_2)}$. The results given in Table XIX show that there is no significant difference in the correlations for the straight grade and patent flour by either the simple or bromate formula. However, those between protein and loaf volume by the malt and bromate formulas are significant, confirming the observations of Larmour (6, 7) in this connection.

TABLE XVIII

SIMPLE CORRELATIONS BETWEEN PROTEIN AND LOAF VOLUME OBTAINED ON 55% PATENT AND STRAIGHT GRADE FLOUR BY DIFFERENT BAKING FORMULAS

Grade	Simple formula		Bromate formula		Blend formula	Malt formula	Approx. value of r at 5% pt.
	Straight flour (*a*) r_{6a}	55% Patent flour (*c*) r_{6c}	Straight flour (*e*) r_{6e}	55% Patent flour (*g*) r_{6g}	55% Patent flour (*k*) r_{6k}	Straight flour (*m*) r_{6m}	
1° and 2°	+ .469	+ .358	+ .599	+ .534	+ .310	+ .692	.27
3°	+ .509	+ .354	+ .674	+ .604	+ .634	+ .695	.32
No. 4	+ .304	+ .332	+ .820	+ .678	+ .572	+ .661	.28
No. 5	+ .620	+ .597	+ .671	+ .707	+ .670	+ .746	.28
No. 6	+ .541	+ .581	+ .621	+ .549	+ .666	+ .598	.31
All grades	+ .460	+ .425	+ .605	+ .571	+ .526	+ .627	.13

The analysis of covariance for wheat protein and various measures of baking quality is given in Table XX and shows that the correlation within grades is somewhat higher than the total. The correlation between grades, which

TABLE XIX

SIGNIFICANCE OF THE DIFFERENCE BETWEEN CORRELATIONS FOR PROTEIN WITH LOAF VOLUME (ALL GRADES COMBINED) OBTAINED BY DIFFERENT BAKING FORMULAS

	Simple correlations		$\frac{Z_1 - Z_2}{\sigma_{(Z_1-Z_2)}}$	5% Point
	Straight flour	Patent flour		
Simple formula	+ .460	+ .425	0.34	1.64
Bromate formula	+ .605	+ .571	0.56	1.64
Simple formula Bromate formula	+ .460 + .605		2.17	1.64
Simple formula Bromate formula		+ .425 + .571	2.05	1.64
Simple formula Malt formula	+ .460 + .627		2.53	1.64
Simple formula Blend formula		+ .425 + .526	1.38	1.64

expresses the relation between the mean protein and mean baking quality for different grades, is insignificant. To illustrate this more clearly the grade means for wheat protein and loaf volumes by the bromate formula straight flour are given at the foot of Table XX. The mean protein content for the various grades is practically constant, yet there is a wide range in the mean loaf volumes. The variation in mean loaf volume with an approximately constant mean protein is a reflection of the differences in the average protein quality and other factors affecting strength of the various grades.

This is very important from the standpoint of grain grading. Since all grades in 1928 had approximately the same mean protein content, this factor obviously could not have been used to advantage as a basis for differentiating between the grades, but rather as an index of quality within each grade. The variability in baking within the grades is apparently due in large part to the variations in protein content.

TABLE XX

ANALYSES OF COVARIANCE FOR WHEAT PROTEIN AND VARIOUS MEASURES OF BAKING QUALITY

Simple correlation coefficients for	Wheat protein and loaf volume						Wheat protein and computed baking score for blend formula		5% Points	Degrees of freedom
	Simple formula, 55% patent flour		Bromate formula, straight flour		Blend formula, 55% patent flour					
	r_{6e}	t	r_{6e}	t	r_{6k}	t	r_{6L}	t		
Total, all grades	+ .425	7.1	+ .605	11.4	+ .526	9.3	+ .536	9.5	1.96	226
Between grades	− .043	0.1	− .014	0.0	− .274	0.5	− .230	0.4	3.18	3
Within grades	+ .440	7.2	+ .674	13.5	+ .561	10.0	+ .584	10.6	1.96	218

Grade	1° and 2°	3°	No. 4	No. 5	No. 6
Mean wheat protein, %	12.2	11.8	12.1	12.0	12.3
Mean L. V. (cc), bromate formula (straight flour)	552	534	557	516	477

The Relation Between Protein Content, Classes of Visible Damage and Baking Quality

The relation between protein content, classes of visible damage, and baking quality is expressed by the simple, partial and multiple correlation coefficients for the entire series in Table XXI. In these calculations, loaf volume by the bromate (patent flour), blend, and malt formulas, and baking score by the blend formula have been taken as representative of baking quality. The simple correlations involving wheat protein and the various classes of kernels show, as might be surmised from a knowledge of the biochemical changes in the maturation of the wheat kernel, that the protein content of wheat is not

TABLE XXI

SIMPLE, PARTIAL AND MULTIPLE CORRELATIONS SHOWING THE RELATION BETWEEN PROTEIN CONTENT, CLASSES OF VISIBLE DAMAGE AND VARIOUS MEASURES OF BAKING QUALITY

	Simple correlation coefficients					Fourth order partial correlation coefficients			
	Wheat protein (6)	L.V. pat. bromate (g)	L.V. pat. blend (k)	L.V. str. malt (m)	Bak. score pat. blend (L)	Loaf volume 55% patent bromate formula (g)	Loaf volume 55% patent blend formula (k)	Loaf volume straight malt formula (m)	Baking score 55% patent blend formula (L)
Total sound (1)	− .016	+ *.268*	+ *.158*	+ *.258*	+ *.263*				
Bran frost (2)	− .000	− *.176*	− *.149*	− *.243*	− *.224*	$r_{2g.3456}$ − .097	$r_{2k.3456}$ − .122	$r_{2m.3456}$ − *.258*	$r_{2L.3456}$ − *.193*
Heavy frost (3)	+ *.135*	− *.167*	− .033	− .036	− .067	$r_{3g.2456}$ − *.245*	$r_{3k.2456}$ − .074	$r_{3m.2456}$ − .076	$r_{3L.2456}$ − .100
Immature (4)	− *.172*	− *.195*	− *.161*	− *.266*	− *.248*	$r_{4g.2356}$ − .110	$r_{4k.2356}$ − .069	$r_{4m.2356}$ − *.196*	$r_{4L.2356}$ − *.169*
Green (5)	+ .056	− .123	− .074	− .091	− *.132*	$r_{5g.2346}$ − .103	$r_{5k.2346}$ − .057	$r_{5m.2346}$ − .037	$r_{5L.2346}$ − .087
Wheat protein (6)		+ *.571*	+ *.526*	+ *.627*	+ *.536*	$r_{6g.2345}$ + *.599*	$r_{6k.2345}$ + *.526*	$r_{6m.2345}$ + *.635*	$r_{6L.2345}$ + *.543*
	At 5% pt. $r = .132$					At 5% pt. $r = .132$			

MULTIPLE CORRELATIONS

$R_{g(23456)} = .643$ $\quad R_{k(23456)} = .557$ $\quad R_{m(23456)} = .692$

$R_{L(23456)} = .609$

At 5% pt. $R = .320$

related in any degree to the individual forms of visible damage. The simple correlations reveal that the best single index of baking quality is protein content, the next being the percentage of total sound kernels, which, however, is a much less reliable measure. The simple correlations between the various classes of damage are low and for the most part insignificant, and indicate that individually they are of little or no value as grading factors.

The fourth order partial correlations show the relation between protein, percentage bran frost, heavy frost, immature, and green kernels respectively on baking quality, when each factor varies alone. It will be observed that none of the individual forms of damage have any appreciable effect on baking quality when the other classes of damage and protein are held constant. In view of these correlations it would seem that in a series of samples differing inherently in quality, apart from damage, the assumption that baking quality can be estimated by the amounts of individual kinds of damage is based on a false analogy to the depreciation in quality found when varying percentages of different forms of damaged kernels are added to samples of a single lot of sound wheat. The relation between protein content and the various measures of baking quality when all forms of damage or in other words, the percentage of total sound kernels, are held constant is, however, quite significant.

It is apparent from the multiple correlations involving the same factors, the largest of which is 0.69, that all the variables have not been accounted for. This is probably due in part to variations in the inherent quality of the sound kernels.

The data show that in so far as baking quality is concerned, the various forms of damage do not require individual consideration in grading and that protein content and percentage of sound kernels are the best single indices of quality.

These conclusions are based on the results for the entire series and there seemed a possibility that the relations of protein and the different classes of visible damage to baking quality might be quite different for the lower grades. Fourth order partials were therefore calculated for grades No. 5 and No. 6 using the baking score for the blend formula as a measure of baking quality. The results given in Table XXII again show that the individual forms of damage are slight and approximately equally correlated with baking quality when varied alone, and that protein content is the most reliable single measure of the baking quality of low grade wheats. The general conclusion is hence the same, whether we consider the entire series collectively or the low grade wheats as a group, that protein content, and the percentage of sound kernels are the best single measures of baking quality.

TABLE XXII

FOURTH ORDER PARTIAL CORRELATION COEFFICIENTS SHOWING THE RELATION BETWEEN PROTEIN CONTENT, CLASSES OF VISIBLE DAMAGE AND BAKING SCORE (BLEND FORMULA) FOR GRADES NOS. 5 AND 6 COMBINED

L = Baking score, blend formula		
2 = % Bran frost	$r_{L2\ 3456}$ = −	305
3 = % Heavy frost	$r_{L3\ 2456}$ = −	302
4 = % Immature	$r_{L4\ 2356}$ = −	393
5 = % Green	$r_{L5\ 2346}$ = −	207
6 = % Wheat protein	$r_{L6\ 2345}$ = +	638

At 5% pt. r = .205

Discussion

The results of this study based on representative samples of the 1928 wheat crop of Western Canada show that the grading system in use that year gives a correct indication of the average relative milling and baking quality of the grades except in the case of No. 4. Although there was a progressive decrease in mean flour yield as the grade decreased, the average baking quality of No. 4 wheat was distinctly superior to that of 3°, and in fact even to grades 1° and 2° combined. It must be recalled that only nine samples grading 1° were included in this study. Wheat quality depends on both the yield and strength of flour and it seems probable that, from the viewpoint of the European miller, the greater strength of No. 4 is more than sufficient to offset its lower flour yield. This anomaly in the relative quality of these grades is not now likely to occur in view of the amendment to the Canada Grain Act in 1930 requiring 3° to contain 25% of hard red vitreous kernels.

In the Canadian grading system, weight per measured bushel and percentage of hard red vitreous kernels are the chief factors used in assigning the statutory grade, while the relative amounts of the various classes of damage present in the samples are paramount in deciding the commercial grades of frosted wheat. The progressive change in the character of the damage in the different commercial grades indicates that the system is applied in a very efficient manner by the Grain Inspection Division.

The data presented here strongly indicate that weight per measured bushel is as valuable an index of flour yield in the commercial as in the statutory grades. Individually, bran frosted, heavily frosted, immature and green kernels do not show a close relation to wheat quality and hence an estimation of the amount of any one particular form of damage is of little value as a grading factor. Collectively, however, they exert an appreciable influence on both milling and baking quality. This implies that the percentage of total damage, or conversely the percentage of sound kernels present, forms the most logical basis for the grading of frosted wheat into the commercial grades.

The adoption of weight per bushel and the percentage of sound kernels as the chief factors in assigning the commercial grades to frosted wheat is an extension of the principles employed in the statutory grades and would thus lead to a unification of the entire grading system. The various classes of sound kernels were grouped together in this preliminary study, in order to simplify the statistical treatment and discussion of the data. In most instances, the percentage of piebald and starchy kernels was not great and there was a close relation between the percentages of total sound and vitreous kernels. Several calculations not reported here were made in which the percentage of vitreous kernels was substituted for "total sound" and the correlations with milling and baking quality were slightly higher. For the sake of uniformity, it would seem desirable to use the percentage of vitreous kernels as a grading factor throughout.

With the exception of the anomalous relationship in the strength of 3° and No. 4 wheats, the grading system gives a satisfactory indication of the

average relative quality of the grades. The variability in flour yield and baking quality within each grade, particularly in the instance of the commercial grades, however, is high, and thus the system fails to give an accurate evaluation of the quality of the individual samples. The high variability within grades is of particular importance to the producer who is interested in the grading of his own comparatively small lot of wheat and the securing of the highest grade to which its quality will entitle it.

While variations in the percentage of sound kernels are in part responsible for the differences in quality within each grade the protein content of the wheat is a much more important factor. The data clearly show that within each grade protein is the most reliable single index of strength, this statement applying equally to the higher and the lower grades. There is, however, no relationship between the mean protein content of the different grades and the average baking quality, implying that, in general, the grades are clearly differentiated on the basis of protein "quality". This is indicated by the decrease in mean response to the differential baking tests with lowering wheat grade.

Since the magnitude of the response is in part related to the percentage of total damage present and the percentage of protein is not related to the classes of kernels, protein content of wheat could not logically be used as a primary grading factor. The ideal grading system, apart from an actual determination of milling and baking quality, would appear to be a primary subdivision into wide groups on the basis of weight per measured bushel and the percentage of hard red vitreous kernels, followed by subclassifications within each group on the basis of protein content.

Since experimental evidence has been accumulating in recent years confirming the conclusion that the protein content of wheat is a good indication of its baking strength and value for blending with weaker wheats, an inquiry has recently been conducted by Newton (12) regarding the feasibility of using protein content as a factor in grading and marketing Canadian wheat. In general, the European purchasers of Canadian wheat discounted the value of the protein test because it indicated quantity rather than quality; the outstanding requirement of the European trade being constancy of grade qualities. In view of the general satisfaction of the European trade with the present grading system and the practical difficulties which would be encountered in the grading, storage and transportation of the crop if protein were utilized as a grading factor, it was not considered feasible at the present time.

The data presented here indicate that constancy of grade qualities in relation to baking behavior cannot be expected with wide variations in protein content, but some improvement in this respect may be effected by reducing variations in quality which depend in part on the percentage of sound kernels present. The recent revision of the Canada Grain Act which raised the percentage of hard red vitreous kernels in the different grades may be expected to bring about a greater uniformity in grade characteristics and the changes made are in line with the results of this study. Owing to the variable nature of wheat and the complexity of the characteristics to be evaluated, it is doubtful whether

it will ever be possible to devise a grading system which will indicate accurately the value of every sample and at the same time be of practical application.

In conclusion, the authors wish to point out that the experimental material on which this study was based was collected in only one crop year and is therefore representative of only one general class of frost damage. In that year, 1928, frost occurred when the wheat was nearly mature, and extended into areas which do not usually produce frosted wheat. The annual surveys now in progress may be expected to show to what extent the various forms of frost damage are correlated with wheat quality in other years.

Acknowledgments

The authors are indebted to the Canadian Co-operative Wheat Producers Limited who extended their facilities in securing the samples and to the Board of Grain Commissioners for the work done by the Inspection Branch and the Grain Research Laboratory. They are deeply indebted to Dr. C. H. Goulden, Cerealist, Dominion Rust Research Laboratory, Winnipeg, for his counsel and advice in connection with the statistical reduction of the data. They also wish to acknowledge the aid of the technical assistants in the three collaborating laboratories.

References

1. American Association of Cereal Chemists. Methods for the analysis of cereals and cereal products. Lancaster Press Inc., Lancaster, Pa. 1928.
2. Blish, M. J. Cereal Chem. 5: 158-161. 1928.
3. Fisher, R. A. Statistical methods for research workers. 3rd ed., Oliver and Boyd, London. 1930.
4. Geddes, W. F. Can. J. Research, 1: 528-558. 1929.
5. Geddes, W. F. and Binnington, D. S. Cereal Chem. 5: 215-220. 1928.
6. Larmour, R. K. Cereal Chem. 7: 35-48. 1930.
7. Larmour, R. K. Cereal Chem. 8: 179-189. 1931.
8. Larmour, R. K. and MacLeod, A. G. Sci. Agr. 9: 477-490. 1929.
9. Larmour, R. K. and MacLeod, A. G. Sci. Agr. 10: 1-22. 1929.
10. Larmour, R. K., Machon, F. D. and Brockington, S. F. Cereal Chem. 8: 233-241. 1931.
11. Malloch, J. G. and Cook, W. H. Cereal Chem. 7: 307-310. 1930.
12. Newton, R. Bulletin No. 14. National Research Council of Canada. 1930.

THE EFFECT OF STORAGE AT VARIOUS MOISTURE CONTENTS ON BAKING QUALITY OF MARQUIS WHEAT[1]

By R. K. Larmour[2]

Abstract

Marquis wheat, in 5-lb. containers, at moisture contents ranging from 10 to 22%, was stored at 21°C. and at outside winter temperature for periods of one and four months. With the exception of samples having 16, 18, 20, and 22% moisture, stored at 21°C. for four months, no evidence either of improvement or deterioration of baking quality was observed. The exceptions noted were lower in quality due principally to mustiness of the wheat.

Introduction

In some seasons in Western Canada harvesting is followed by a period of wet weather that delays threshing very considerably and results in appearance on the market of tough and damp wheat. Tough wheat contains over 14.4% and not more than 17.0%, and damp wheat contains more than 17.0% moisture. Wheat falling in either of these categories cannot be graded "straight" but is designated "no grade". The proportion of this "no grade" wheat is very large in some years. For the crops produced in 1925, 1926, and 1927, the percentages of this class of wheat were 28.6, 50.8, and 44.7 respectively. The limits set for tough wheat are based upon observations of the moisture content at which wheat may be stored without danger of damage from heating. While the main problem with this class of wheat has been to ascertain exactly the optimum safe condition for drying, there must also be considered the question whether or not the wetting and subsequent storage, under conditions that preclude heating, have any effect on the baking quality of the wheat. Wheat that has been tough or damp and subsequently dried to below 14.4% moisture often exhibits a marked change in appearance, the color becoming dull and "bleached" and the endosperm, as shown when the kernel is cut, changing from a clear vitreous to a soft white starchy appearance. There is thus a change in aspect of the grain from that of hard wheat to soft wheat and because this weathered wheat looked soft there was held, to some extent, the opinion that its quality had changed to that of soft wheat. It seems impossible, however, that the mere addition and removal of water should have so profound an effect as to change the inherent quality.

This problem was investigated by the Associate Committee on Grain Research, National Research Council of Canada (2), in the course of a study on the drying of wheat that had been exposed to varying degrees of weathering under known conditions. Some samples of damp wheat were stored in steel

[1] *Manuscript received December 21, 1931.*
Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Canada, with financial assistance from the Saskatchewan Agricultural Research Foundation. Issued as Paper No. 30 of the Associate Committee on Grain Research, National Research Council of Canada.

[2] *Associate Professor of Chemistry, University of Saskatchewan.*

drums at outside temperature during the winter and then dried, milled and baked. The results obtained were not as decisive as might have been desired due to the fact that the prolonged exposure of the grain in the stook was favorable to sprouting and it was thought that this may have occurred to some extent. This of course would tend to obscure the effects of storage. There were observed a number of cases in which there was evidence of improvement and a number in which no apparent change took place. It could not be definitely determined whether or not the improvement were due to traces of sprouted grain and, therefore, the only conclusion reached was that "winter storage of damp sound wheat in steel drums of 40-80 gallons capacity, in no case resulted in significant deterioration of quality, and in some cases apparent improvement took place." The fact that those samples showing no change in quality had been stored at a moisture content lower than the others suggested that there might be a quite definite moisture limit below which no improvement could be expected. In order to investigate this question the work hereinafter described was undertaken.

Materials and Methods

Ten 20-lb. lots from a uniform sample of unweathered No. 2 Northern Marquis of 13.6% protein content were made up to moisture contents of 10, 11, 12, 13, 14, 15, 16, 18, 20 and 22% respectively. These moisture levels will hereinafter be designated by the numbers 1-10 respectively. These lots were then each subdivided into four 5-lb samples which were placed in cans just large enough to contain that quantity and the lids sealed thoroughly with paraffin. Two of the four series thus obtained were stored in a constant temperature room at 21°C. and the other two were placed outside on the north of the building. The experiment was started on December 1, so that those placed outside froze immediately and remained frozen during the term of storage.

After one month one of the series stored inside and one stored outside were opened, and those samples above 13% moisture were dried rapidly at room temperature by exposing them on mesh-bottomed trays over which a current of air was passed by means of an electric fan. They were then tempered and milled in the usual manner. The remaining two series were opened at the end of four months and treated similarly. The following designation of these series will facilitate reference to them:

Series I—No. 1-10 inclusive—Stored inside for one month
Series II—No. 1-10 inclusive—Stored outside for one month
Series III—No. 1-10 inclusive—Stored inside for four months
Series IV—No. 1-10 inclusive—Stored outside for four months

All samples except Nos. 9 and 10 of Series III appeared to be in good condition at the time the cans were opened. These two however were badly molded and quite out of condition. There was no evidence of heating in any of the samples stored at 21°C. The flours, milled to a 55% patent, were placed in cotton sacks immediately after milling and permitted to age for one

month, after which time they were transferred to half-gallon glass fruit jars and kept well sealed. Bakings were made in duplicate by the simple and bromate formulas using 50 gm. of flour and the procedure described by Larmour and MacLeod(1). In a second and third baking there was used, in addition to the simple and bromate, a blend formula involving the bromate formula applied to a mixture of 40% soft flour and 60% of the flour under examination. This will be referred to as the blend-bromate formula. These flours were baked after aging for one month, which means that Series III and IV (stored four months) were baked three months after Series I and II (stored one month). Then six months after the last baking, all forty samples were re-baked. At this time Series I and II had aged ten months and Series III and IV had aged seven months. Three months after this they were all baked again. As all three bakings showed the same general results, the average values were calculated and will be used in the following discussion.

Discussion of Results

Comparison of the four series may be made on the basis of the loaf volume and the baking score. For the sake of brevity the other bread scores have been omitted as they show nothing that is not to be found in these two.

TABLE I

COMPARISON OF LOAF VOLUME AND BAKING SCORE OF THE FOUR SERIES BAKED BY THE SIMPLE FORMULA

Sample No.	Moist. at which stored, %	Series I, inside, one month	Series II, outside, one month	Series III, inside, four months	Series IV, outside, four months
Loaf volume, cc.					
1	10	278	280	279	290
2	11	288	279	276	285
3	12	271	276	280	286
4	13	283	277	279	281
5	14	272	273	279	277
6	15	279	281	277	274
7	16	278	276	279	279
8	18	280	281	254	283
9	20	285	273	305	278
10	22	278	278	344	274
Baking score					
1	10	79	83	80	83
2	11	83	82	77	80
3	12	76	80	77	82
4	13	82	83	76	79
5	14	76	79	79	77
6	15	78	83	78	78
7	16	78	81	82	79
8	18	80	82	68	78
9	20	84	82	83	78
10	22	80	83	84	83

There was practically no change in texture, crumb color or general appearance of the loaves in the series, except in the case of Nos. 9 and 10, Series I, stored at 21°C. for four months with 20 and 22% moisture respectively. These samples were quite out of condition due to molding and gave bread of very poor color and texture. The loaf volumes and baking scores by the simple formula are given in Table I. These data show no evidence of any significant changes that might be attributed to differences in moisture content except in the case of No. 8, (moisture content, 18%), stored four months at 21°C. This sample was distinctly lower than the others in both loaf volume and baking score and it may be concluded that some damage to quality occurred with this treatment. Comparing the different series, there appears to be no significant differences between those stored at 21°C. and those stored at below 0°C. either for one month or for four months, nor does the difference in length of storage seem to have produced any definite changes.

The simple formula which involves use of flour, yeast, sugar, salt, and water only, is not very satisfactory for experimental flours, because it frequently fails to bring out the real strength and therefore often leads to false conclusions. For the purpose of evaluating wheat strength it is advisable to use some sort of improver or oxidizer in order to "loosen up" the gluten and permit the

TABLE II

COMPARISON OF LOAF VOLUME AND BAKING SCORE OF THE FOUR SERIES BAKED BY THE BROMATE FORMULA

Sample No.	Moist. at which stored, %	Series I inside, one month	Series II, outside, one month	Series III, inside, four months	Series IV, outside, four months
Loaf volume, cc.					
1	10	339	336	332	337
2	11	344	329	340	339
3	12	328	342	339	345
4	13	343	349	337	343
5	14	329	335	336	343
6	15	335	338	326	330
7	16	337	341	326	334
8	18	338	348	304	343
9	20	341	334	308	340
10	22	328	329	315	338
Baking score					
1	10	117	117	114	117
2	11	119	114	117	116
3	12	112	120	115	118
4	13	117	121	115	118
5	14	113	119	114	118
6	15	115	119	111	114
7	16	114	118	112	115
8	18	116	121	101	118
9	20	118	117	97	115
10	22	111	115	88	116

development of optimum hydration and elasticity. In this study, however, the simple formula was used in order to see if the treatments given the samples would show evidences of modification of the gluten. Saunders, Nichols, and Cowan (3) reported that successive wettings and dryings of wheat caused a change in the wheat which resulted in improved baking properties when a formula similar to the simple formula was used. It was thought that keeping the wheat damp for as long a period as four months might produce somewhat similar results. The data however fail to show any tendency toward improvement.

The results obtained by use of the bromate formula are given in Table II. The values were remarkably constant except in the cases of Nos. 8, 9, and 10, Series III, samples with moisture contents of 18, 20 and 22% respectively and stored at 21°C. for four months. In these samples there was undoubtedly lowering of both loaf volume and baking score. The 18% sample on inspection showed no evidence of damage but it was unmistakably lower in baking quality than the preceding members of Series III (stored inside for four months). It should be noted here that the following two members of this series, which did not show evidence of damage when baked by the simple formula, were by the bromate formula distinctly lower in quality than the

TABLE III

COMPARISON OF LOAF VOLUME AND BAKING SCORE OF THE FOUR SERIES BAKED BY THE BLEND-BROMATE FORMULA

Sample No.	Moist. at which stored, %	Series I, inside, one month	Series II, outside, one month	Series III, inside, four months	Series IV, outside, four months
Loaf volume, cc.					
1	10	299	300	296	306
2	11	310	298	299	300
3	12	302	305	298	290
4	13	314	303	295	302
5	14	298	303	297	301
6	15	305	306	296	305
7	16	301	296	285	299
8	18	309	307	277	300
9	20	305	296	268	301
10	22	302	300	306	293
Baking score					
1	10	96	97	94	98
2	11	100	92	92	95
3	12	94	96	93	92
4	13	100	97	92	95
5	14	93	98	93	95
6	15	97	99	94	97
7	16	94	93	87	95
8	18	99	97	82	95
9	20	98	92	73	95
10	22	95	95	76	91

upper members of the series. All the other samples exhibit variations no greater than might be expected in replicate bakings of one sample of flour.

A blend of soft flour with the flour under investigation baked by the bromate formula frequently brings to light differences in quality not apparent from the data obtained by either the simple or bromate formula. In Table III however the only additional fact revealed by this blend-bromate formula was that sample No. 7, as well as Nos. 8, 9, and 10, Series III, was low in quality. No. 10, Series IV, which was stored at below 0°C. with 22% moisture was slightly lower in loaf volume and baking score than those immediately preceding it in Series IV, but this cannot be considered significant because No. 3 stored with only 12% moisture showed nearly identical values.

Summing up the results of the baking tests by all three formulas, only the samples with 16, 18, 20 and 22% moisture, stored at 21°C. for four months, showed evidence of deterioration. All the others exhibited differences no greater than would be expected from replicate bakings of one flour. This indicates that if wheat at moisture content up to 22% can be stored at or below 21°C. in quantities small enough to avoid heating no measurable change in quality will occur in one month. If the wheat can be kept at below 0°C. (32°F). no change occurs during a period of four months. When the moisture exceeds 15%, samples stored at 21°C. (70°F.) for four months undergo deterioration in quality, the extent of which increases with the moisture.

References

1. Larmour, R. K. and MacLeod, A. G. Sci. Agr. 9: 477-490. 1929.
2. National Research Council of Canada. Report No. 24. 1929.
3. Saunders, C. E., Nichols, R. W. and Cowan, P. R. Can. Exptl. Farm Bull. 97. 1921.

THE TRIAENOPHORUS PARASITE IN THE FLESH OF THE TULLIBEE (LEUCICHTHYS)[1]

By Daniel Nicholson[2]

Abstract

In the flesh of many tullibee and some whitefish there are yellow cysts which contain a thread-like worm 5 to 25 cm. (2 to 10 in.) in length. As these fish are favorite foods, a study of the characteristics and pathogenicity of any larvae found in their flesh is important. This paper describes the appearance of the parasite and relates it to its adult form in the intestine of the jackfish (*Esox lucius*) which preys on young tullibee and white fish. Feeding the parasite to dogs did not result in the development of adult tapeworms and from this it is inferred that triaenophorous parasite is non-pathogenic to man.

Incidence of Triaenophorus Infestation

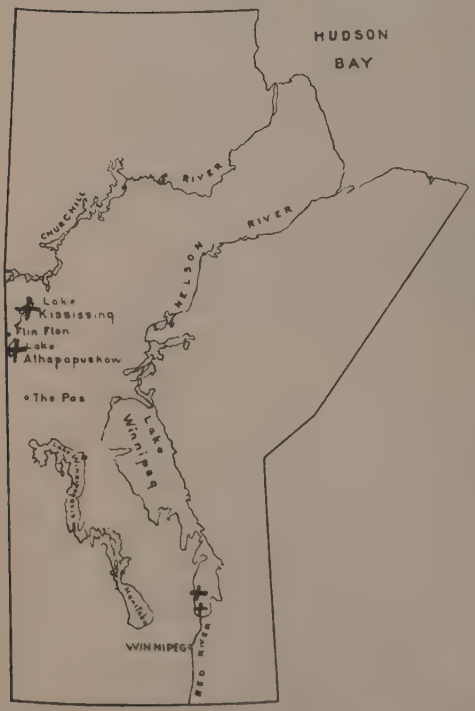


Fig. 1. *Map of Manitoba Lakes. The tullibee examined were caught in the localities marked with a cross.*

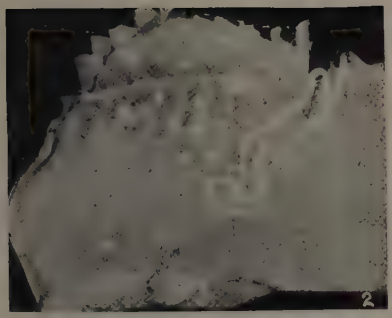
The first lot of 48 tullibee examined were caught in Lake Winnipeg during September 1929. They were identified by Dr. A. Bajkov as *Leucichthys nigripinnis* and *Leucichthys tullibee*. Forty-one were infested, each containing from 2 to 18 parasites. In another lot of 60 tullibee caught during May 1920, 46 were infected, each containing from one to ten parasites.

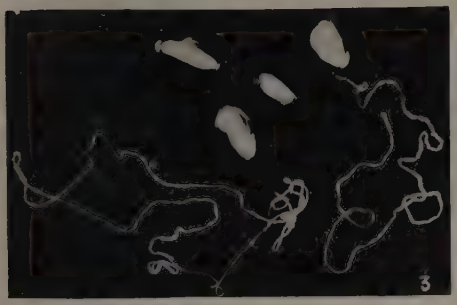
Out of 23 whitefish (*Coregonus clupeaformis*) caught during January 1931 in Kississing Lake, which is north of the Pas, 18 were infected with triaenophorus parasites. Of twenty whitefish caught in Lake Athapapuskow about the same time only one was infected with triaenophorus.

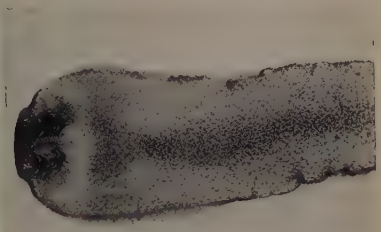
There are five varieties of tullibee in Lake Winnipeg and from extensive investigation of the incidence of infestation and feeding habits of each of these

[1] *Manuscript received July 14, 1931.*
Contribution from the Department of Pathology, University of Manitoba, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada.

[2] *Assistant Professor of Pathology, University of Manitoba. Member of the Scientific Advisory Board to the Fisheries Branch, Department of Natural Resources, Province of Manitoba.*







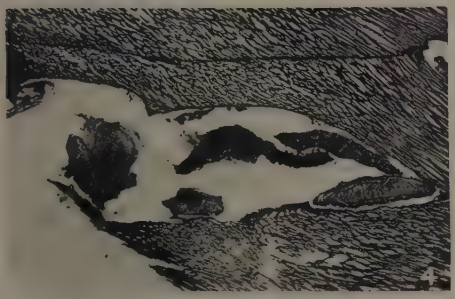


FIG. 2. *Slice of tullibee showing a large active triaenophorus parasite which has crawled out of the opened cyst just below the coils. The white spots on the fish are drops of bichloride used to arrest the movement of the parasite for purposes of photography. Natural size ×0.7.*

FIG. 3. *Thick walled cysts containing triaenophorus (above) and long slender triaenophorus (below) contained in such cysts. Natural size ×0.7.*

FIG. 4. *A section through a cyst showing the surrounding muscle, a thin cyst wall and some sections of the parasite. The fluid contents of the cyst have drained away. ×6.*

FIG. 5. *Section through the wall of a cyst showing the character of the contents above and the fish muscle below. There are no bacteria or pus cells present in this exudate. ×130.*

FIG. 6. *Head of a large triaenophorus larval parasite from tullibee. The head is slightly pressed between two glass slides and turned up to show the hooklets on both sides. ×9.*

FIG. 7. *Head of adult triaenophorus tapeworm from intestine of the pike. It shows the resemblance to the head of the larval form in Fig. 6. The hooklets on the posterior side show through just above and to the right of the hooklets on the proximal side. ×19. Reproduced from paper cited (2).*

species, Dr. Bajkov finds that the black backed tullibee (*L. tullibee*), of which 60% are infected with triaenophorus parasites, feed mainly on the bottom fauna of the lakes, together with some of the surface plankton. The black fin (*L. nigripinnus*), the small bloater (*L. hoyi*), and the black back nipigon tullibee (*L. nipigon*), are almost 100% infected and they feed mainly on plankton which is near the surface. The long jaw (light back) tullibee, (*L. zenithicus*) feeds on bottom fauna and is very lightly infected. About 25% contain one, and rarely more than two, parasites. The whitefish, which is closely related to the long jaw (light back) tullibee and also a bottom feeder, is slightly infected. About 10% of them contain a few parasites. The breeding of the light back tullibee in hatcheries would tend to lessen infestation.

Morphology of the Triaenophorus Parasite

To examine the fish, it is placed on its side, the skin slit longitudinally near the dorsal fin and again on the lateral side of the belly. The skin can then be peeled off from head to tail. With a long, sharp knife thin slices are pared off.

In a few, the cysts containing the triaenophorus are thin-walled and transparent. These always contain plump, active parasites. In most fish the cyst wall is yellowish and moderately tough; this type of cyst frequently contained a yellowish milky fluid surrounding the parasite.

Microscopic examination of this fluid showed it to be amorphous material as shown in Fig. 5. No tissue cells or bacteria were present. The triaenophorus in this type of cyst was slender and much coiled and many of them had no movement. This would suggest that the cyst wall developed from irritation by the parasite. The triaenophorus parasite ranges in length from 5 to 25 cm. (2 to 10 in.) and in diameter from 1 to 3 mm. The body is round or ovoid and the segments are indistinctly marked in contrast to the adult tapeworm form. Most of the parasites were quite active and crawled entirely out of the cyst when it was opened up. The head showed a very definite structure with two trident shaped hooklets on each side (Fig. 6.) The similarity of this to the head of the tapeworm found in the intestine of the pike is very striking (*cf.* Fig. 7).

Feeding Experiments to Determine the Effect of the Triaenophorus on Mammals

As it is very important to determine whether these parasites are detrimental to mammals, the author prepared three dogs by examining the feces for ova and parasites before and after dosing with felix mas. This being negative, the dogs were then fed slices of fish containing triaenophorus parasites. One dog was fed 12, another 18 and a third, 24 parasites. After this they were fed wholly on biscuits for a month and they thrived well. One month after the feeding, chloroform was administered and a necropsy performed. There were no tape or round worm parasites in the intestinal tract. The other

internal organs and the skeletal muscles were carefully examined by making thin slices and inspecting under a hand lens. No lesions or parasites could be seen.

In May 1930, three dogs were again subjected to feeding experiments using technique similar to that in the previous test, except that the dogs were fed more parasites. Twenty-four were fed to one dog, 36 to the second and 48 to the third. Post-mortem examination one month later revealed no intestinal parasites or infestation of the internal organs or skeletal muscles. The fact that the larval form of the triaenophorus parasite did not cause infestation in dogs would indicate that other mammals including man were not capable of being infested.

Other Parts of the Triaenophorus Life Cycle

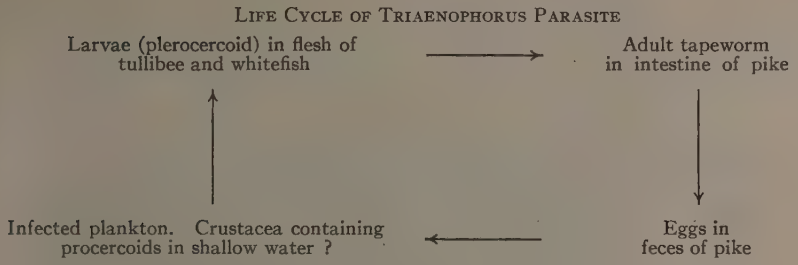
(*a*) *As an Adult Tapeworm in the Intestine of the Pike*

During 1927 and 1928 while examining pike (*Esox lucius*) for parasites (*Diphyllobothrium latum*), the author was surprised to find that the intestine of the pike contained many small tapeworms having two broad, trident-shaped hooklets on each side of the head. The body possessed well-marked segments and the mature end segments contained a uterus from which tiny eggs could be extruded on pressing the segment between two glass slides. This parasite was identified by Dr. Bajkov as a triaenophorus and a photograph of the head was included in the article then published on fish tapeworm. It is reproduced here in Fig. 7, to show its similarity to the parasite found in the flesh of the tullibee.

The jackfish or pike (*Esox*) is sometimes called the fresh water shark. It preys on other fish, frequently on tullibee and whitefish. If these are infested with triaenophorus larvae, the identical appearance of the heads and hooklets would suggest that the small adult tapeworm in the intestine of the pike grows from the larval form in the flesh of the devoured tullibee. The segments in the adult worm are more distinct and flatter than in the larval forms and the ripe terminal ones extrude eggs.

(*b*) *Food of Tullibee and Whitefish as a Link in the Life Cycle*

The steps in the life cycle are unknown from the extrusion of the triaenophorus egg with the feces of the pike to the infestation of the tullibee by the larval plerocercoid. Food is the most likely source of infestation and the tullibee and whitefish do not eat other fish but live entirely on the plankton and bottom fauna, the minute forms of animal life that abound in lake waters. If the plankton become infested by ingesting triaenophorus eggs or their embryos, they would be the most likely link in the infestation of the tullibee. From his extensive investigations of the food of various species of fish Dr. Bajkov is of the opinion that some of the small crustaceans probably, *Limnocalanus macrurus*, *Epischura lacustris*, *Diaptomus scilis* or *Mysis relicta* may be the intermediate hosts between the triaenophorus egg extruded in the feces of the pike and the larval form in the flesh of the tullibee.


Life Cycle of Triaenophorus Parasite
Larvae (plerocercoid) in flesh of tullibee and whitefish
Adult tapeworm in intestine of pike
Infected plankton. Crustacea containing procercoids in shallow water ?
Eggs in feces of pike

Conclusions

1. The adult tapeworm in the intestine of the pike resembles the larval plerocercoid in the flesh of the tullibee and likely develops through the pike eating infested tullibee.

2. The triaenophorus parasite which infests the flesh of tullibee and whitefish is non-pathogenic to dogs and likely also to all mammals. Its appearance is objectionable.

3. The practical difficulties of clearing the lakes of jackfish (*E. lucius*) should be carefully investigated. This fish is the commonest host of Triaenophorus and Diphyllobothrium parasites. It eats other valuable fish and itself has little market value. A bounty for *Esox* caught up creek at spawning time or an industry that could utilize them for fish meal or fertilizer might be considered.

Acknowledgments

The author is much indebted to Dr. Alexander Bajkov for identifying the varieties of fish and in giving him the benefit of his wide experience in the incidence of infestation and feeding habits of tullibee and whitefish. Dr. Moorhouse and Dr. Ormerod, Department of Physiology, University of Manitoba,very kindly gave their facilities for the dog feeding experiments.

References

1. Bajkov, A. Biological conditions of Manitoban lakes. Contr. Can. Biol. Fisheries, v.5 no. 12. 1930.
2. Nicholson, D. Fish tapeworm. Intestinal infection in man: the infestation of fish in Manitoba lakes. Can. Med. Assocn. J. 19: 25-33. 1928.

DIPHYLLOBOTHRIUM INFECTION IN *ESOX LUCIUS*[1]

By Daniel Nicholson[2]

Abstract

This study showed that most of the summer caught jackfish or pike (*Esox lucius*) contained many larvae of *D. latum* (plerocercoids), in contrast to the winter caught fish which showed a scanty infestation. The larvae in the winter caught fish were small and frequently motionless, while those in the summer caught fish were large and quite active.

When dogs were fed *motile* larvae from winter caught fish, very few adult tapeworms developed. Sixty per cent of the larvae contained in the summer caught fish produced adult tapeworms. This suggests that *D. latum* infection of the *Esox* fish is probably an annual event occurring in the early summer months.

It is the small to medium sized *Esox* that contain numerous *D. latum* parasites. The very large fish contained so few parasites as to suggest that after a period of development the *D. latum* larvae (plerocercoid) in the flesh of the fish die off and disintegrate leaving no trace of their presence, and as the fish become large the procercoid form of the parasite has difficulty in penetrating the intestinal wall of the fish.

Larvae in Summer Caught Esox

The larvae in the flesh of summer caught *Esox* are very numerous. Most of them measure $\frac{1}{2}$ to $\frac{3}{4}$ in. in length and have some of the characteristics of mature tapeworms, such as the slit head and rudimentary segments. They are quite active and when placed on the wetted surface of the hand, crawl about vigorously. Even after ordinary cold storage freezing, the larvae when warmed exhibit this activity.

Larvae in Winter Caught Esox

There are very few larvae (plerocercoids) in the flesh of *Esox* caught during March, April and May, as shown in Table I. Those found are very small and many are degenerated so that when moved with a probe they break up. Of those that remained intact on probing only 9% showed movement, which was quite sluggish in character. In those that are motile the segments are quite rudimentary and the slit in the head is only moderately developed.

The findings in Table I would suggest that infection of *Esox* with *D. latum* parasites takes place in the summer and that the parasites die off after five to six months.

Infestation in Large Fish

Fifty-two per cent of the *Esox* 10 to 18 in. in length harbored parasites which were frequently very numerous. As many as 63 were counted in one fish. It was very striking that only a few of the *Esox* over 24 in. long harbored parasites, nor was there the slightest macroscopic trace of past infestations. Classified from the standpoint of size, only 3% of the fish over 24 in. long harbored parasites and then only one or two small parasites were found.

[1] *Manuscript received August 13, 1931.*
Contribution from the Department of Pathology, University of Manitoba, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada.

[2] *Assistant Professor, Department of Pathology, University of Manitoba, and Member of the Scientific Advisory Board to the Fisheries Branch, Department of Natural Resources, Province of Manitoba.*

TABLE I

DEGREE OF INFESTATION OF *Esox* CAUGHT IN LAKE WINNIPEG, AUGUST 1928 TO JUNE 1931

Date	Number examined	No. containing larvae	Degree of infestation	State of larvae	Date	Number examined	No. containing larvae	Degree of infestation	State of larvae
1928					1930				
Aug.	48	43	C	X	Jan.	60	21	B	YZ
Sept.	36	35	C	X	Feb.	84	12	AB	YZ
Oct.	24	21	C	X	Mar.	120	3	A	Z
Nov.	24	19	B	XY	April	96	2	A	Z
Dec.	60	34	B	XZ	May	96	6	B	Y
					July	24	19	C	XY
1929					Aug.	30	28	C	X
Jan.	72	21	AB	YZ	Oct.	12	12	C	X
Feb.	80	17	A	YZ	Nov.	18	15	C	XY
Mar.	96	0	O		Dec.	24	14	BC	YZ
Apr.	120	6	A	YZ					
Aug.	30	28	C	X	1931				
Sept.	18	18	C	X	Jan.	36	13	BA	YZ
Oct.	18	16	C	X	Feb.	60	10	BA	YZ
Nov.	36	31	C	XY	Mar.	72	4	A	Z
Dec.	48	37	CB	Y	Apr.	60	3	A	Z
					May	44	5	AB	Y
					June	32	9	B	Y

NOTE:—*Degree of infestation: O = No larvae; A = 1 to 3 larvae; B = 3 to 12 larvae; C = over 12 larvae. State of larvae: X = Very active, large; Y = Sluggishly active, small; Z = No motion, some degenerated.*

It is hardly reasonable to suppose that these large fish have been free from parasites in all the previous summers of their existence. Moreover the intestinal wall of large *Esox* must offer more resistance to the procercoid of the *D. latum* larvae than the intestinal wall of the smaller fish. They are both caught in the same waters and their stomach contents are similar. All this is considerable evidence that the infestation of *Esox* by *D. latum* parasites is not permanent.

Feeding Experiments to Determine Viability of Parasites

To test the ability of the parasites to develop into adult tapeworms three dogs were fed with *motile* larvae of *D. latum* from winter caught *Esox*. One was given 5, another 10 and the third 15 larvae from fish caught during January and February 1929. Before infesting the dogs they were carefully examined and dosed with felix mas to make sure that they harbored no parasites. After eating the larvae the dogs were fed wholly on biscuits and water for a period of a month. A post-mortem examination was then made. The dog that had eaten five motile larvae of *D. latum* had one adult tapeworm (*D. latum*). The second dog that had eaten 10 larvae showed 5 adult tapeworms and the one that had eaten 15 showed 4. Thus only 27% of the motile larvae from winter caught fish when fed to dogs developed into adult tapeworms.

The experiment was repeated in August 1930, feeding the larvae from summer caught fish to dogs according to the routine already outlined. One

dog ate five large vigorous larvae from summer caught fish and developed four tapeworms (*D. latum*). Another ate 10 summer larvae and developed 6 tapeworms. A third ate 15 larvae and developed 8 tapeworms. These dogs lost weight rapidly toward the end of the experiment. Thus 60% of the larvae from summer caught fish developed into tapeworms.

The infestation from winter caught fish is really much lower than the above experiment would suggest when one remembers that very few winter caught fish are infested, and of the few larvae found only an occasional one shows any motility, and of the ones showing motility only 27% developed into adult tapeworms. This is quite in agreement with the views of Indians and fishermen around Lake Winnipeg who say that winter caught jackfish (*Esox*) are not harmful to their dogs but that if the latter are fed on jackfish caught in summer, they will develop worms. During the latter part of the summer it is not an uncommon sight around Lake Winnipeg to see dogs that have parasites protruding from the rectum, and this would suggest that dogs are the most important mammalian link in the life cycle of the *D. latum* parasite. Many of the eggs from the parasites in dogs are fertile and when incubated develop into swimming embryos.

Temperatures Required to Kill Parasites

Batches of 12 summer caught fish were rolled in a paper and placed in cold storage at varying temperatures for 24 hr. When the lowest point registered was −11° C. (+12.2° F.), by a minimum recording thermometer placed among the fish, all parasites were killed. The parasites were not killed in fish that were stored in cold storage for 24 hr. where the minimum temperature recorded in the same manner was −3° C. (+26.6° F.). During these experiments no record was kept of the rapidity with which the minimum temperature was reached. In all experiments the minimum temperature recorded was present when the fish were removed. If many fish are packed closely, the low temperature takes longer to penetrate than when fish are in a small package. Rapid changes in temperatures are more fatal to life than gradual changes and the duration of the low temperature is important. Further experiments should be conducted to find out the time necessary to lower the temperature in the centre of a large pile of boxes of fish before this measure could be adapted for commercial purposes. While the cooking of fish kills all parasites, should the *Esox* or the *Leucioperca vitreum* (pickerel) be objected to on the ground that they harbor dangerous parasites, this objection could be overcome by chilling to −11° C. for 24 hr.

Discussion

The pike which were the subject of this study were caught in Lake Winnipeg, which is about 240 miles long, extending from a southern latitude of 50.5° in a northern and slightly western direction to a latitude of 53.7°. The north end of the lake has a longer and colder winter season and its surrounding country is sparsely inhabited. All except the catch of August, September and October 1928 were taken from the southern arm of the lake which forms a large bay almost 100 miles long. The country surrounding this part of the lake is well

settled and the sewage from the City of Winnipeg, some 40 miles distant, drains by way of the Red River into the south end of the lake.

It was not possible to obtain fish from a single area, although it would have been ideal for this study. The fish examined were those supplied for sale in the open market and the author was informed by Mr. Roundtree of the Winnipeg Fish Company that many of the summer caught fish are obtained near the shore while the winter caught fish are usually taken through the ice farther off shore. Most of the summer caught fish were taken from the vicinity of Matlock. Dr. Alexander Bajkov has informed the author that there is a lagoon near Gimli in which *Esox* are more heavily infested even in the winter than are the fish caught in the other parts of the lakeshore. He attributes this to the stillness of the water, the many dogs around this area which infest the water with ova of *D. latum*, and the fact that the water is shallow and warmer than in the main lake.

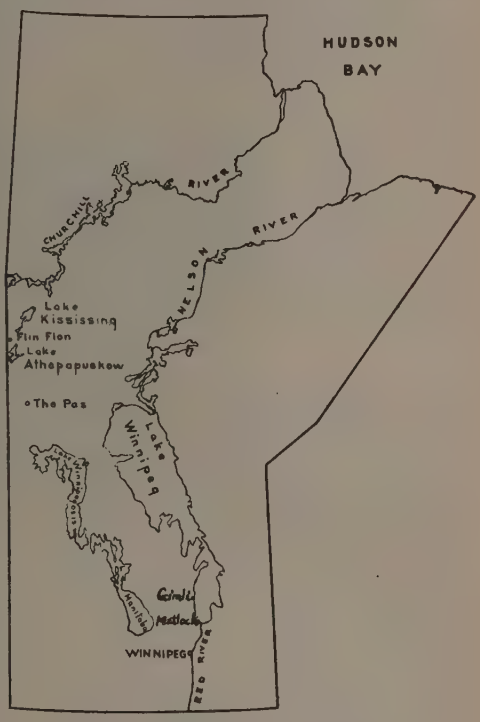


FIG. 1. *Map of Lake Winnipeg.*

During the winter, low temperatures kill ova deposited on excreta on the ice and the water is too cold for any of the embryos to hatch from eggs that might be brought in by running streams or sewage.

In April and May the water warms sufficiently to allow ova of *D. latum* to develop into embryos. These embryos are eaten by diatoms which in turn are ingested by the fish.

Conclusions

1. The numerous large active plerocercoid larvae of *D. latum* found in *Esox* during the summer, when contrasted with the infrequent, inactive and sometimes degenerated larvae found during the winter and spring, suggest that the source of infestation is more prevalent during the summer months.

2. The parasite after living in the muscle for several months, shrivels up and degenerates. Large fish rarely harbor parasites.

3. The fishing of *Esox* which are to be marketed should be done during the late winter and spring months and summer fishing prohibited.

4. Freezing to −11° C. will kill the larvae of *D. latum* in the *Esox*.

5. As dogs are an important link in the infestation, those harboring parasites should be dosed with malefern and advice given to their owners regarding the prevention of further infestation. Dogs should not be fed on raw summer caught fish.

6. As the *Esox* has little or no market value, means of getting rid of it in lakes should be considered. A bounty might be offered for fish caught up creeks at spawning time or it would be well to consider an industry that could make use of them for fishmeal or fertilizer. This would also break the life cycle of the triaenophorus parasite which inhabits the intestines of the *Esox* as a tapeworm, and spends its larval stage in the flesh of tullibee, whitefish and other species that have good market value.

Acknowledgments

The author is indebted to the late Mr. Arthur Rigby, food inspector, Winnipeg Health Department and to Mr. G. N. Rowntree, Manager of the Winnipeg Fish Co., for supplying fish together with information regarding their origin. Dr. V. H. K. Moorhouse and Dr. M. J. Ormerod, Department of Physiology, University of Manitoba, kindly gave facilities for the dog feeding experiments.

References

1. Bajkov, A. Biological conditions of Manitoban lakes. Contr. Can. Biol. Fisheries, v. 5, no. 12. 1930.
2. Nicholson, D. Fish tapeworm. Intestinal infection in man: the infestation of fish in Manitoba Lakes. Can. Med. Assocn. J. 19: 25-33. 1928.
3. Nicholson, D. Variations in the fish larvae of *Diphyllobothrium latum*. Can. Public Health Journal. 20: 193-195. 1929.

INHERITANCE OF RESISTANCE TO FOWL PARALYSIS (*NEUROLYMPHOMATOSIS GALLINARUM*)

I. DIFFERENCES IN SUSCEPTIBILITY[1]

By V. S. Asmundson[2] and Jacob Biely[3]

Abstract

Data on the incidence of paralysis and lymphomatous tumors in a flock of 542 pullets of six different breeds indicate the presence of both in 14 out of 52 paralyzed pullets, while an additional 22 had tumors but were not paralyzed. The association of paralysis and tumors may have been due to chance. The evidence presented, while not conclusive, points to the inheritance of resistance to paralysis. This is indicated particularly by (1) differences in the proportion of paralyzed pullets in different breeds, and (2) absence of paralysis among the progeny of certain males and in certain large families. The data obtained point to a simple mode of inheritance.

Among the important problems facing the poultry farmer is the control of disease. Since it is now generally accepted that resistance to disease depends, in some cases at least, upon hereditary factors, it follows that one of the methods of controlling losses from disease is to breed resistant strains. Considerable progress along this line has been made with plants. Much research has also been conducted on the inheritance of disease resistance in animals and the breeding of disease resistant strains of domestic animals has been started.

The three projects with domestic fowls that have, so far, been reported on are: (*a*) Inheritance of resistance to diphtheria (5); (*b*) Inheritance of resistance to fowl typhoid (6, 7); and (*c*) Inheritance of resistance to pullorum disease (1). Frateur (5) found evidence that resistance to diphtheria was determined by a single dominant factor. Lambert and Knox (6, 7) hatched chicks from unexposed parents and from parents that had survived an acute infection of fowl typhoid. The mortality among chicks from unexposed parents was 41% as compared with a mortality of 90% in the case of chicks from unexposed parents. They consider that resistance is inherited on the basis of multiple factors. The data of Card and Roberts (1) show fairly clearly that resistance and susceptibility to pullorum disease are, at least in part, due to the existence of hereditary factors. In addition to these reports Mathews (8) found some evidence "that heredity is associated with the occurrence of leukochloroma." Mathews and Walkey (9) have presented evidence in support of the theory "that the lymphoid neoplasms are inherited as a mendelian recessive characteristic."

As yet, no attempt has been made to study the inheritance of resistance to fowl paralysis, although it is generally recognized that the widespread and frequent outbreaks of paralysis cause tremendous losses to the poultry industry.

[1] *Manuscript received December 3, 1931.*
Contribution from the Departments of Poultry Husbandry and Bacteriology, University of British Columbia, Vancouver, Canada, with financial assistance from the National Research Council of Canada.

[2] *Associate Professor, Department of Poultry Husbandry, University of British Columbia.*
[3] *Research Assistant, Department of Poultry Husbandry, University of British Columbia.*

Pappenheimer, Dunn and Cone (12) report that mortality may range from a few per cent up to 60% and over. Furthermore, the disease recurs year after year.

Pappenheimer *et al.* (12) noted what they called a "very significant indication of a breed or varietal difference in susceptibility to paralysis." They state that 14 out of 27 Silver Spangled Hamburg chicks died from paralysis, while other chicks kept under the same conditions and at the same time, did not develop paralysis.

Doyle (2, 3) has obtained evidence which, he considers, shows that fowl paralysis is hereditary. He found that chicks hatched out of eggs from flocks in which paralysis was present have a tendency to develop paralysis.

Thomas (13) states that fowl paralysis is transmitted with great difficulty and that a hypothesis of individual susceptibility, or alternately, general natural resistance, has been advanced to explain this.

Experimental

The data reported in this paper are from a flock of 542 pullets comprising six breeds. These had been hatched and reared together. A few cases of paralysis occurred before the pullets were banded and removed to the laying houses, but all those banded were apparently in good health. The losses in this case thus occurred somewhat later than usual. The clinical and pathological symptoms, which will be described in detail in a subsequent paper, have been found to correspond with those described by Pappenheimer *et al.* (12), Doyle (2, 3), Dobberstein and Haupt (4), Thomas (13), McGaughey and Downie (10).

Pappenheimer *et al.* presented evidence in favor of the view that the association of fowl paralysis with visceral lymphomata, originating in the ovary, is not accidental, and that the lymphomata are a manifestation of the disease. The data for this flock that have a bearing on this problem are shown in Table I.

TABLE I

DISTRIBUTION OF PARALYSIS AND LYMPHOMATOUS TUMORS BY BREEDS

Breed	No. of pullets in families free from tumor and paralysis	No. of pullets in families in which paralysis or tumors were present			
		Normal	Paralysis only	Tumors only	Paralysis and tumors
S.C.R.I. Reds	49	51	13	2	4
B.P. Rocks	64	25	4	3	1
White Wyandottes	33	21	7	2	
White Wyandottes*		50	2	6	3
Australorps	34	17	2	1	
Light Sussex	21	3			1
S.C.W. Leghorns	39	35	10	8	5
Total	240	202	38	22	14

**Unpedigreed birds from flock matings.*

It will be seen from this table that, of the 516 birds for which some information is available (on 25 birds no post-mortem examination was made), 52 were paralyzed and 36 had tumors. Fourteen of these had tumors and were paralyzed. On the basis of the total population of 516 pullets, the number of cases where both occur together is 3.8 times the number expected as a result of chance. Presumably the 240 pullets in families free from paralysis and lymphomatosis were resistant and may, on that assumption, be not considered. When the calculation is based on the remaining 276 pullets belonging to families or groups in which some paralysis or tumors occurred, the ratio becomes 2:1. The simultaneous occurrence of tumors and paralysis in these 14 birds may, therefore, be due to chance. Accordingly, only the cases of actual paralysis are included in Table II, but it should be stated that the interpretation of the data in this table would not be materially changed by grouping the birds having lymphomata with the paralyzed birds.

TABLE II

SUMMARY OF ALL FAMILIES OF PULLETS HATCHED IN 1930 SHOWING INCIDENCE OF PARALYSIS

Sire No.	All of pullet progeny normal		Part of pullet progeny paralyzed			Died, no post-mortem
	No. of dams (families)	No. of pullets kept	No. of dams (families)	No. of pullets kept		
				Normal	Paralysis	
1[1]	2	6	3	10	7	3
2[1]	1	4	3	19	6	1
3[1]	4	18	2	16	2	1
4[1]	3	15				
5[1]	2	10	2	4	2	
6[2]	3	3	1	1	1	
7[2]	7	23				
8[2]	6	26	1	3	1	1
9[2]	2	8	2	10	3	2
10[2]	5	13				
11[2]	2	5				
12[3]	2	9	6	23	7	5
13[3]	7	24				5
14[4]			?	56*	5*	1
15[5]	4	23	1	12	1	
16[6]			1		1	
17[5]	4	17				3
18[6]	4	19				
19[6]	1	2	1	3	1	
20[7]	4	10	4	13	5	
21[7]	2	4				1
22[7]	2	9	2	3	2	
23[7]	5	20	2	2	2	2
24[7]	4	12				
25[7]	1	1	4	4	4	
26[7]			2	4	2	
Totals		281		127	47	25

1—S.C.R.I. Reds; 2—B.P. Rocks; 3—White Wyandottes; 4—White Wyandottes from a flock mating; 5—Australorps; 6—Light Sussex; 7—S.C.W. Leghorns.

**Not included in totals.*

Incidence of Paralysis

The losses sustained from paralysis among the progeny of different males, grouped according to breed, are shown in Table II. This table is based on cases occurring after the end of September when the birds were about six months old. Since on 25 birds no post-mortem examination was made and others were sent to market during the latter part of the year, this table may not show the total possible cases of paralysis. Accurate information is, however, available for the period of heaviest losses from paralysis, hence there is every reason to believe that it shows the relative incidence of paralysis fairly accurately. On account of the small number of pullets in any one family it was not considered advisable to present the details for each family.

There was a difference in the proportion of paralyzed pullets of the various breeds. The percentages in each breed were as follows:

Australorps, 3.7%; Light Sussex, 4.0%; B.P. Rocks, 5.2%; W. Wyandottes, 9.7%; S.C.R.I. Reds, 14.3%; S.C.W. Leghorns, 15.5%.

Since the number of birds, in the case of some of these breeds, was relatively small, it cannot be assumed that real breed differences are involved, but the variation in the percentage of birds affected may indicate differences in the strains and groups here used.

There appears to be a difference in susceptibility to paralysis among the progeny of different families (Table II). Unless there is a difference in susceptibility, it is difficult to account for the absence of paralyzed pullets among the daughters of some of the males. This might be due to chance in the case of males Nos. 11 and 21, since there were only five and four daughters respectively, but it is not likely that chance alone is responsible for the absence of paralyzed pullets among the progeny of males Nos. 4, 7, 10, 13, 17, 18 and 24. The proportion of paralyzed to normal daughters from other males was 1:6. (47 paralyzed to 276 normal). The proportion in families where some paralysis occurred was higher or approximately 1 paralyzed to 3 normal (47 paralyzed to 127 normal, Table II). Assuming on this basis and for the sake of simplicity that one-fourth of the progeny of males Nos. 4, 7, 10, 13, 17, 18 and 24 would have become paralyzed if chance alone governed the results, there should have been from three to six paralyzed daughters from each of these males. The deviation from 3:1 ratio, the probable error, Dev. P.E. and odds against these deviations being due to chance for the progeny of these seven males are as shown in Table III.

TABLE III

Male No.	No. of progeny	Dev.	P. E.	Dev./P.E.	Odds
4	15	3.75	1.13	3.3	37.4:1
7	23	5.5	1.37	4.0	142.3:1
10	13	3.25	1.05	3.1	26.4:1
13	24	6.00	1.43	4.2	215.8:1
17	17	4.25	1.24	3.4	44.8:1
18	19	4.75	1.27	3.7	78.5:1
24	12	3.0	1.01	3.0	22.2:1

The odds against these deviations from an expected 3:1 ratio being due to chance alone vary from 22:1 to 216:1. Some allowance should perhaps be made for the fact that the proportion of paralyzed daughters among the progeny of other males was less than one-fourth. Even if this is done and a further allowance made for deaths from undetermined causes among the progeny of males Nos. 13 and 17 (see Table II), the evidence here presented is such as to indicate that the absence of paralysis in the progeny of these seven males is not due to chance alone. These results, therefore, point to inherent differences as an important factor in resistance to paralysis. In further support of this conclusion it is of interest to note that there were several families comprising 7 to 11 pullets, none of which became paralyzed, although pullets from the same sire but a different dam were affected.

Mode of Inheritance

Considering next the possible basis of inheritance of resistance to paralysis, it will be noted (Table II) that slightly over one-fourth of the pullets in these families became paralyzed. Assuming a single factor difference, this proportion of affected pullets would indicate that the parents were heterozygous for the gene for susceptilibity to paralysis. Since this was apparently the first time that the flock had been exposed to paralysis, some of the parents would be expected to be susceptible and, as a result, half or all of their progeny would be susceptible, depending upon the constitution of the other parent. An examination of the individual families shows that in about 12 families the number of paralyzed and non-paralyzed pullets is equal, thus pointing to a 1:1 ratio. All of these are small families of not more than five pullets. There were, in addition, three families of one pullet each which were paralyzed, but the numbers here are too small to furnish critical evidence. The data indicate that resistant birds differ from susceptible birds by a single dominant gene, but since other factors besides heredity may influence the incidence of paralysis, no conclusion can, in the present state of knowledge, be drawn with respect to this point. If the mode of inheritance of resistance to paralysis is simple it should be a relatively easy matter to breed strains of domestic fowl that are highly resistant to paralysis. The greatest obstacle at present is the difficulty of reproducing the disease under laboratory conditions. Further work on these phases of the problem is now in progress.

References

1. Card, L. E. and Roberts, E. Proc. Fourth World's Poultry Congress (Lond.). Sec. C. 526-533. 1930.
2. Doyle, L. P. J. Am. Vet. Med. Assocn. 68, n.s. 21: 622-630. 1926.
3. Doyle, L. P. J. Am. Vet. Med. Assocn. 72, n.s. 25: 585-587. 1928.
4. Dobberstein, J. and Haupt, H. Z. Infectionskrankh. Haustiere, 31: 58-80. 1927.
5. Frateur, J. L. Proc. Second World's Poultry Congress (Barcelona). Sec. 1. 68-71. 1924.
6. Lambert, W. V. and Knox, C. W. Iowa State College, J. Sci. 2: 179-187. 1928.
7. Lambert, W. V. Sci. Monthly, 28: 118-121. 1929.
8. Mathews, F. P. Arch. path. 7: 442-457. 1929.
9. Mathews, F. P. and Walkey, F. L. J. Cancer Research, 13: 383-400. 1929.

10. McGaughey, C. A. and Downie, A. W. J. Compt. Path. Therap. 43: 63-76. 1930.
11. Pappenheimer, A. M., Dunn, L. C. and Cone, V. C. J. Exptl. Med. 49: 63-86. 1929.
12. Pappenheimer, A. M., Dunn, L. C. and Cone, V. C. Storrs Agr. Exptl. Sta. Bull. 143: 187-290. 1926.
13. Thomas, A. D. J. S. African Vet. Med. Assocn. 67: 178-183. 1928.

STUDIES ON B.C.G. VACCINE

II. NON-VIRULENCE AND RESISTANCE IN NEW-BORN CALVES

By A. C. Rankin[2], J. J. Ower[3], R. M. Shaw[4],
P. R. Talbot[5] and the late H. M. Vango[6]

Abstract

Bovines fed B.C.G. shortly after birth do not show tuberculous lesions, nor any evidence of tuberculous infection at autopsy two years after such vaccination. This is demonstrated in animals vaccinated by mouth with suitable controls. Unvaccinated unprotected controls living closely in contact with these vaccinated animals were at the end of the same period quite free from tuberculous infection. Such evidence supports the contention that there is no return of virulence in B.C.G. in the animal body and that, therefore, vaccinated animals are not a source of danger to unprotected animals.

In a previous paper by the authors (3) attention was drawn to the resistance to tuberculous infection in animals vaccinated with B.C.G., and to the non-pathogenicity of B.C.G., grown according to Calmette's methods, in subcutaneously vaccinated calves over periods of one year.

It was also pointed out that animals vaccinated subcutaneously with B.C.G. exhibited a marked resistance to subsequent intravenous inoculations with virulent bovine bacilli, as compared with non-vaccinated controls, which confirmed the results of experiments carried out by Calmette and Guerin (2). These experiments were continued with the purpose of estimating the resistance to natural infection of calves vaccinated by mouth, and particularly of establishing the virulence or non-virulence of B.C.G. vaccine fed to new-born calves. The methods employed in the maintenance of the culture, preparation of the vaccine, etc., were those recommended by Calmette, and similar to those followed by the authors in their previous work (3). The culture used was received from Calmette in 1925, and since that time had been maintained on glycerinated potato and synthetic broth with occasional transfer to bile potato medium.

During August, September, and October 1928, 76 new-born calves were purchased in the neighborhood of Edmonton and brought together in a specially prepared barn on the outskirts of the city, where it was considered that they would be free from possible sources of tuberculous infection. These animals, with a few exceptions, were secured from dairy herds tested for tuberculosis,

[1] *Manuscript received January 11, 1932.*
Contribution from the Provincial Laboratories, University of Alberta, Edmonton, Alberta, with financial assistance from the National Research Council of Canada. These studies form part of a co-operative attack on the problem of tuberculosis, carried on jointly by the National Research Council of Canada, the Dominion Department of Agriculture, Fort Qu'Appelle Sanatorium, McGill University, Queen Alexandra Sanatorium, Queen's University, the Sun Life Assurance Company of Canada, the University of Alberta, the University of Montreal and the University of Toronto.

[2] *Dean of the Faculty of Medicine and Professor of Bacteriology and Hygiene at the University of Alberta, and Chairman of the Committee on Tuberculosis Research of the Province of Alberta, of which Drs. Ower, Shaw, Talbot and Vango were members.*
[3] *Professor of Pathology and Provincial Serologist, University of Alberta.*
[4] *Professor of Bacteriology and Hygiene, University of Alberta.*
[5] *Professor of Veterinary Science, University of Alberta.*
[6] *Late Associate Professor of Medical Jurisprudence and Assistant Professor of Pathology, University of Alberta.*

immediately after birth, in most cases even before they had been fed. They were, to the best of the authors' ability, protected from possible sources of tuberculous infection and were fed on pasteurized milk. Half of the number were vaccinated by mouth with three doses of B.C.G. vaccine (50 mg. moist weight) in milk on the third, fifth and seventh day subsequent to birth, the vaccine being composed of the bacilli from glycerinated potato or synthetic broth, and not more than 21 days old.

Mouth vaccination might be considered somewhat similar to such vaccination in children, though certainly not identical on anatomical and physiological grounds. As the organism is of bovine strain, its pathogenicity should manifest itself in the calf. The vaccinated animals and the controls though kept separated at first, in the barn and pasture, were under the same living conditions and were fed adequately for normal growth and maintenance*. During the months of September, October and November 1928, eight of these young animals died of disease other than tuberculosis, leaving at the end of the year, 68 animals, 34 of which had been vaccinated and 34 controls.

In March 1929, these animals were divided into the Edmonton and Ponoka groups, each consisting of 17 controls and 17 vaccinated animals. One group constituted the material for Experiment No. 1, 1928-30, an investigation of the virulence of the organism for new-born calves, and the other was used in Experiment No. 2, 1928-30, which was concerned with the resistance of calves vaccinated by mouth to contact infection. In the same month the animals of Experiment No. 2 were placed in contact with a tuberculous herd and fed on milk from tuberculous cows.

TABLE I

RESULTS OF EXPERIMENT NO. 1, 1928–1930, ON SIXTEEN QUARANTINED UNVACCINATED CONTROLS

No. of animal	Sex	Date of birth 1928	Date of slaughter 1930	Time elapsed, months	Post-mortem findings	
					Macroscopic	Microscopic
276	F	August 25	October 23	26	Negative	Negative
277	M	August 28	October 22	26	Negative	Negative
278	M	August 29	October 23	26	Negative	Negative
279	M	September 7	October 20	25	Negative	Negative
280	M	September 7	October 20	25	Negative	Negative
281	M	September 9	October 23	25	Negative	Negative
282	M	September 8	October 23	25	Negative	Negative
283	M	September 12	October 23	25	Negative	Negative
285	M	September 13	October 23	25	Negative	Negative
286	M	September 13	October 23	25	Negative	Negative
288	M	September 13	October 20	25	Negative	Negative
289	M	September 13	October 22	25	Negative	Negative
292	M	September 22	October 22	25	Negative	Negative
293	M	September 22	October 20	25	Negative	Negative
296	F	September 24	October 22	25	Negative	Negative
300	M	October 2	October 22	24	Negative	Negative

On the advice of Professor J. P. Sackville, Professor of Animal Husbandry, University of Alberta.

TABLE II

RESULTS OF EXPERIMENT NO. 1, 1928–30, ON SIXTEEN QUARANTINED ANIMALS VACCINATED BY MOUTH

No. of animal	Sex	Date of birth 1928	Date of vaccination 1928	Date of slaughter 1930	Time elapsed, months	Post-mortem findings	
						Macroscopic	Microscopic
251	M	August 17	August	October 20	26	Negative	Negative
252	M	August 15	August	October 22	26	Negative	Negative
253	M	August 22	August	October 20	26	Negative	Negative
254	M	August 24	August	October 20	26	Negative	Negative
255	M	August 26	August	October 20	26	Negative	Negative
258	M	August 29	August	October 20	25	Negative	Negative
259	F	August 29	August	October 20	25	Negative	Negative
261	M	August 30	August	October 20	25	Negative	Negative
263	M	August 30	August	October 20	25	Negative	Negative
264	M	September 1	August	October 20	25	Negative	Negative
265	M	September 1	August	October 20	25	Negative	Negative
267	M	September 3	August	October 20	25	Negative	Negative
268	M	September 4	August	October 20	25	Negative	Negative
269	F	September 5	August	October 20	25	Negative	Negative
270	F	September 6	August	October 20	25	Negative	Negative
271	M	September 11	August	October 20	25	Negative	Negative

Investigation of Virulence

EXPERIMENT NO. 1, 1928-30

The Edmonton Group

In June 1929, animal No. 291 (a control) died of pneumonia, and in March 1930, animal No. 241 (vaccinated) was slaughtered because of its poor condition, leaving 32 animals in this experiment, 16 vaccinated and 16 controls.

In June 1929 the animals were allowed to run in a common pasture in which, as far as could be ascertained, there had been no animals for several years and which could, therefore, reasonably be considered to be non-infected. The pasture, which included bush, was enclosed by a wire fence to limit the movements of the animals to the non-infected area. Feed was supplied, the animals eating out of a common trough, and drinking Edmonton city water, also from a common trough. The controls and vaccinated animals therefore lived together, eating and drinking from the same supply of food and water, and being subject to the same possibilities of acquiring tuberculous infection. All feed, hay and grass were bought on the open market and did not come from protected sources.

On several occasions during the experiment, particularly in the spring of 1930, the animals broke through the retaining wire and were some hours at large in the neighborhood, where, however, there were no other cattle. These were, of course, breaks in the quarantine, but would not appear to have interfered with the experimental results as they have been subsequently recorded.

These animals passed the winter of 1929-30 together, mostly out of doors, and were at the time of slaughter over two years of age. They were slaughtered in Gainer's Packing Plant, Edmonton, on October 20-22 under careful observation, and while Federal Government inspectors were present. The whole

group of animals was passed for food, as there was no evidence of tuberculosis.

Glandular material taken at these autopsies was injected into guinea pigs and prepared for microscopic examination. In Tables I and II are shown the results of the examination of the tissues of these bovines.

The results of the microscopic examination of the glandular material from these animals confirm the macroscopic, and in the cases of control animals Nos 282 and 283, in which the macroscopic findings were slightly suspicious, demonstrated in one case actinomyces, and in the other a condition due to Gram positive branching filaments without spores or radial arrangement. Pigs inoculated with material from these lesions were negative to tests for tuberculosis. The tissues of the guinea pigs which were inoculated with glandular material from this group of animals on October 20 and 22, 1930, were examined both macroscopically and microscopically with negative results, and thus there was complete agreement between the three methods of examination. In Tables III and IV, are shown the results obtained.

The results of the experiment show that B.C.G. cultured and administered in the manner indicated is non-virulent for new-born calves. It is also evident that the passage of this organism through the vaccinated calves did not lead to the natural infection of the non-protected controls in close contact. Taken in conjunction with the authors' other experiments, already reported, we think we have very strong evidence indicating the non-virulence for bovines of this organism, carried according to Calmette's method, and we know of no publication in which the contrary is proved, experimentally or otherwise.

From the vaccinated animals of Experiment 2, 1928-30, we can be permitted, we think, to add 14 animals, vaccinated by mouth and which did not exhibit lesions attributable to B.C.G. vaccine, to the 16 mouth-vaccinated animals of this experiment, making in all 30 vaccinated animals quite free from tuberculous infection two years after mouth vaccination.

TABLE III

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED OCT. 20-22, 1930, WITH GLANDULAR MATERIAL FROM 16 QUARANTINED UNVACCINATED CONTROLS OF EDMONTON GROUP, EXPERIMENT 1

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
276	Pig 1	Died April 17, 1931. Acute gastroenteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
	Pig 2	Died April 21, 1931. Acute pneumonic consolidation—lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
277	Pig 1	Died Nov. 16, 1930. Extensive pneumonic consolidation—both lungs. No evidence of tuberculosis. *Micro.*—Purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died March 19, 1931. Extensive pneumonic lesions, acute pleurisy and pericarditis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5

TABLE III—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
278	Pig 1	Died Nov. 14, 1930. Pneumonic consolidation—lungs. No evidence of tuberculosis. *Micro.*—Acute pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died Nov. 19, 1930. Pneumonic lesions—lungs. No evidence of tuberculosis. *Micro.*—Purulent pneumonia. No evidence of tuberculosis.	Not one month
279	Pig 1	Died March 29, 1931. Acute pneumonia and peritonitis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 20, 1931. Healthy pig. *Micro.*—No evidence of tuberculosis.	10
280	Pig 1	Died March 25, 1931. Recently pregnant. Pneumonic lesions in lung. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 20, 1931. Healthy pig. *Micro.*—No evidence of tuberculosis.	10
281	Pig 1	Died Nov. 17, 1930. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died Feb. 12, 1931. Pneumonic consolidation of lung, empyema. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3½
282	Pig 1	(Pus) Died Jan. 1, 1931. Extensive hemorrhagic pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	2
	Pig 2	(Pus) Killed March 30, 1931. Healthy pig. *Micro.*—No evidence of tuberculosis.	5
	Pig 3	Died Feb. 25, 1931. Gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4
	Pig 4	Died March 13, 1931. Extensive pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	4½
283	Pig 1	(Pus) Died Dec. 15, 1930. Extensive hemorrhagic pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	(Pus) Died Feb. 17, 1931. Pig much emaciated. Recently pregnant. No gross evidence of disease. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3
	Pig 3	Died April 21, 1931. Pig very thin. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—Not examined.	6
	Pig 4	Died July 7, 1931. Very thin, recently pregnant. No evidence of disease. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	8½

TABLE III—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
285	Pig 1	Died April 17, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
	Pig 2	Died April 21, 1931. Acute pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
286	Pig 1	Died Nov. 21, 1930. Extensive pneumonic consolidation of lung. Acute pleurisy. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died April 18, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
288	Pig 1	Died Jan. 16, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	2½
	Pig 2	Died March 8, 1931. Extensive pneumonic lesions of lungs. Empyema. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3½
289	Pig 1	Died Nov. 22, 1930. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Killed Aug. 20, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10
292	Pig 1	Died Jan. 12, 1931. Pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	2½
	Pig 2	Died Feb. 19, 1931. Pig very emaciated. Scattered pneumonic foci in lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3
293	Pig 1	Died Feb. 22, 1931. No obvious cause of death. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4
	Pig 2	Died April 9, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
296	Pig 1	Died Dec. 12, 1930. Extensive hemorrhagic pneumonic lesion of lungs. Pig recently pregnant. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	Died Feb. 23, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4
300	Pig 1	Died Dec. 10, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. Acute pleurisy. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	Killed Aug. 27, 1931. Pregnant healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10

NOTE:—*Totals—16 animals, 36 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of hemorrhagic pneumonia and gastro-enteritis in pigs.*

TABLE IV

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED OCT. 20–22, 1930 WITH GLANDULAR MATERIAL FROM SIXTEEN QUARANTINED ANIMALS VACCINATED BY MOUTH. EDMONTON GROUP, EXPERIMENT 1

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
251	Pig 1	Died Dec. 18, 1930. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	2
	Pig 2	Pig ill, killed June 29, 1931. No gross evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	8
252		Not examined.	
253	Pig 1	Died April 9, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died June 2, 1931. A few congested areas in the lungs. No other abnormality. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	7½
254	Pig 1	Died Dec. 12, 1930. Recently pregnant. No gross pathological findings at autopsy. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	1½
	Pig 2	Died March 9, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
255	Pig 1	Died Jan. 19, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3
	Pig 2	Killed Aug. 24, 1931. Recently pregnant, healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10
258	Pig 1	Died Feb. 2, 1931. Pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3
	Pig 2	Died March 30, 1931. Recently pregnant pig. Totally exsanguinated (acute hemorrhage). No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
259	Pig 1	Died Dec. 18, 1930. Suppurative cervical lymphadenitis (smear shows Gram positive bacilli, no tubercle bacilli). Scattered pneumonic foci in lungs. No evidence of tuberculosis. *Micro.*—Suppurative cervical lymphadenitis. Acute bronchial pneumonia. No evidence of tuberculosis.	2
	Pig 2	Died Jan. 15, 1931. Extensive inflammatory lesions in subcutaneous and muscle planes. No evidence of tuberculosis. *Micro.*—Cellulitis (acute). No evidence of tuberculosis.	3
261	Pig 1	Died Nov. 22, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	13
	Pig 2	Died Feb. 26, 1931. Extensive pneumonic consolidation of lungs. Acute pericarditis. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. Acute pericarditis. No evidence of tuberculosis.	4

TABLE IV—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
263	Pig 1	Died Dec. 21, 1930. Recently pregnant. Acute pneumonic consolidation of lungs. Purulent pericarditis. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. Purulent pericarditis. No evidence of tuberculosis.	2
	Pig 2	Died April 13, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
264	Pig 1	Died Dec. 12, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. Acute pleurisy. No evidence of tuberculosis. *Micro.*—Purulent bronchitis, pneumonia and pleurisy. No evidence of tuberculosis.	1½
	Pig 2	Died March 4, 1931. Recently pregnant. No gross pathology of organisms. *Micro.*—No evidence of tuberculosis.	4
265	Pig 1	Pig died Feb. 10, 1931. Extensive pneumonic lesions of lungs. Bilateral plastic pleurisy. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3½
	Pig 2	Died April 19, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
267	Pig 1	Died Feb. 5, 1931. Acute pneumonic consolidation of lungs, with gangrene. No evidence of tuberculosis. *Micro.*—Purulent pneumonia, with gangrene. No evidence of tuberculosis.	3½
	Pig 2	Died March 30, 1931. Recently pregnant, marked pelvic peritonitis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
268	Pig 1	Died Dec. 12, 1930. Extensive pneumonia hemorrhagic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	Died March 15, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
269	Pig 1	Died Nov. 22, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Extensive purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died March 6, 1931. Recently pregnant. Acute endometritis. Broncial pneumonia. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
270	Pig 1	Died Nov. 18, 1930. Extensive pneumonic consolidadation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died Feb. 27, 1931. Pneumonic consolidation of lungs. Recently pregnant. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	4
271	Pig 1	Died Jan. 19, 1931. Pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3
	Pig 2	Died Feb. 26, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4

NOTE:—*Totals—16 animals, 30 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of pneumonia and gastro-enteritis in pigs.*

Investigation of the Resistance to Contact Infection of Animals Vaccinated by Mouth

Ponoka Group EXPERIMENT No. 2, 1928-30

As stated, the animals of this experiment were, in March 1929, placed in contact with a tuberculous herd. For a period of four months they were

TABLE V

RESULTS OF EXAMINATION OF 16 UNVACCINATED CONTROLS PLACED IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2, 1928–30.

No. of animal	Sex	Date of birth 1928	Placed in contact 1929	Time in contact in months	Date of slaughter 1930	Age at slaughter in months	Post-mortem findings	
							Macroscopic	Microscopic
226	M	Sept. 6	March	6+	Oct. 31	25		
227	M	Sept. 7	March	6+	Oct. 31	25		
228	M	Sept. 9	March	6+	Oct. 31	25		Positive
229	M	Sept. 11	March	6+	Oct. 31	25		
231	M	Sept. 12	March	6+	Oct. 31	25	Positive	Positive
233	F	Sept. 13	March	6+	Oct. 31	25		
234	M	Sept. 14	March	6+	Oct. 31	25		
235	F	Sept. 14	March	6+	Nov. 1	25	Positive	Positive
236	F	Sept. 15	March	6+	Nov. 1	25		
237	M	Sept. 17	March	6+	Nov. 1	25	Positive	Positive
238	M	Sept. 18	March	6+	Nov. 1	25		
239	M	Sept. 18	March	6+	Nov. 1	25	Positive	Positive
295	M	Aug. 24	March	6+	Nov. 1	26	Positive	Positive
297	M	Aug. 24	March	6+	Nov. 1	26		
298	M	Aug. 24	March	6+	Nov. 1	26		
299	M	Aug. 25	March	6+	Nov. 1	26		

NOTE:—*Total, 16; positive 6.*

TABLE VI

RESULTS OF EXAMINATION OF 17 ANIMALS VACCINATED BY MOUTH AND PLACED IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2, 1928–30

No. of animal	Sex	Date of birth 1928	Date of vaccination 1928	Placed in contact 1929	Time in contact in months	Date of slaughter 1930	Age at slaughter in months	Post-mortem findings	
								Macroscopic	Microscopic
242	M	Oct. 3	Oct.	March	6+	Oct. 31	25	Positive	Positive
243	M	Oct. 1	Oct.	March	6+	Oct. 31	25		
245	M	Oct. 1	Oct.	March	6+	Oct. 31	25		
246	M	Oct. 1	Oct.	March	6+	Oct. 31	25		
247	M	Sept. 29	Sept.	March	6+	Oct. 31	25		
248	M	Sept. 28	Sept.	March	6+	Oct. 31	25		
249	M	Sept. 27	Sept.	March	6+	Oct. 31	25	Positive	Positive
257	M	Sept. 21	Sept.	March	6+	Oct. 31	25		
272	M	Sept. 11	Sept.	March	6+	Nov. 1	25		
273	M	Sept. 19	Sept.	March	6+	Nov. 1	25		
274	M	Sept. 19	Sept.	March	6+	Nov. 1	25		
275	M	Sept. 20	Sept.	March	6+	Nov. 1	25		
303	M	Oct. 19	Oct.	March	6+	Nov. 1	24		
304	M	Oct. 20	Oct.	March	6+	Nov. 1	24		
305	M	Oct. 20	Oct.	March	6+	Nov. 1	24	Positive	Positive
307	F	Oct. 22	Oct.	March	6+	Nov. 1	24		
308	F	Oct. 28	Oct.	March	6+	Nov. 1	24		

NOTE:—*Total, 17; positive, 3.*

TABLE VII

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED ON OCTOBER 31 AND NOVEMBER 1, 1930 WITH GLANDULAR MATERIAL FROM 16 UNVACCINATED CONTROLS IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
226	Pig 1	Died April 10, 1931. Pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	5½
	Pig 2	Killed Oct. 10, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11½
227	Pig 1	Died April 13, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died April 15, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
228	Pig 1	Died May 2, 1931. Generalized tuberculosis. *Micro.*—Generalized tuberculosis.	
	Pig 2	Died May 21, 1931. Generalized tuberculosis. *Micro.*—Generalized tuberculosis.	
229	Pig 1	Died April 13, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died April 16, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
231	Pig 1	Died Jan. 25, 1931. Tuberculosis of superficial and deep inguinal nodes, lungs, liver and spleen.	
	Pig 2	Died Feb. 12, 1931. Tuberculosis of superficial and deep inguinal nodes, lungs, liver and spleen.	
233	Pig 1	Killed Sept. 14, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
	Pig 2	Killed Sept. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
234	Pig 1	Died Dec. 2, 1930. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	1
	Pig 2	Died March 10, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4
235	Pig 1	Died Feb. 24, 1931. Extensive generalized tuberculosis. *Micro.*—Extensive generalized tuberculosis.	
	Pig 2	Died April 15, 1931. Extensive generalized tuberculosis.	
236	Pig 1	Died April 25, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Killed Aug. 13, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9
237		No pigs inoculated.	

TABLE VII—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
238	Pig 1	Died April 15, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died April 17, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
239	Pig 1	Killed Sept. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
	Pig 2	Killed Sept. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
295	Pig 1	Died Dec. 25, 1930. Extensive generalized tuberculosis.	
	Pig 2	Died Dec. 26, 1930. Extensive generalized tuberculosis.	
297	Pig 1	Died Nov. 24, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died March 3, 1931. Extensive pneumonic consolidation of lungs. Acute pleuritis, pericarditis, peritonitis. Recently pregnant. No evidence of tuberculosis. *Micro.*—Generalized septico-pyaemia. No evidence of tuberculosis.	4
298	Pig 1	Died Dec. 11, 1930. Pneumonic lesions of lungs. Recently pregnant. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died March 10, 1931. Pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	4
299	Pig 1	Died April 15, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Killed Aug. 27, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9½

NOTE:—*Totals—16 animals, 30 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of pneumonia and gastro-enteritis in pigs. Animal No. 228, not macroscopically positive, see Table No. IV.*

fed with milk from tuberculous cattle and on April 10 were placed in close contact with tuberculous cows. This contact was maintained latterly in the open, till November 1, 1929, when they were removed from contact. They passed the winter of 1929-30 in a straw shed, which had been previously used for tuberculous experimental cattle. During the following summer they were again in the open until the time of slaughter. A control animal, No. 232,

TABLE VIII

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED OCTOBER 31 AND NOVEMBER 1, 1930 WITH GLANDULAR MATERIAL FROM 17 ANIMALS VACCINATED BY MOUTH AND PLACED IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
242	Pig 1	Died April 30, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
	Pig 2	Killed Sept. 26, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
243	Pig 1	Died April 13, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 29, 1931. No evidence of tuberculosis.	12
245	Pig 1	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9½
	Pig 2	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9½
246	Pig 1	Died March 19, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
	Pig 2	Killed Oct. 10, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
247	Pig 1	Died April 9, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11½
248	Pig 1	Died Nov. 29, 1930. Pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Killed Oct. 22, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11½
249	Pig 1	Died Dec. 19, 1930. Extensive generalized tuberculosis.	
	Pig 2	Died Dec. 27, 1930. Extensive generalized tuberculosis.	
	Pig 3	(Reinoculated from Pig 2). Killed Feb. 24, 1931. Extensive generalized tuberculosis.	
	Pig 4	(Subinoculated from Pig 3). Died March 30, 1931. Extensive generalized tuberculosis.	
257	Pig 1	Died April 9, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 29, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	12
272	Pig	No record.	
	Pig 2	Killed Sept. 14, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10

TABLE VIII—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
273	Pig 1	Died April 19, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9
274	Pig 1	Died April 9, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
275	Pig 1	Died Nov. 29, 1930. No evidence of tuberculosis. *Micro.*—Acute bronchial pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died April 7, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
303	Pig 1	Died Nov. 28, 1930. Pneumonic foci in lungs. No evidence of tuberculosis. *Micro.*—Bronchial pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died Nov. 28, 1930. Acute purulent pneumonia. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
304	Pig 1	No record.	
	Pig 2	Killed Oct. 22, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
305	Pig 1	Died Dec. 31, 1930. Extensive generalized tuberculosis.	
	Pig 2	Died Jan. 27, 1931. Extensive generalized tuberculosis.	
307	Pig 1	Killed Sept. 14, 1931. Pregnant healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10
	Pig 2	Killed Oct. 10, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
308	Pig 1	Died April 18, 1931. Acute gastro-enteritis. No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9

NOTE:—*Totals—17 animals, 36 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of pneumonia and gastro-enteritis in pigs. Animal No. 242—the small gastric tuberculous gland, upon which the autopsy diagnosis was based, was not inoculated into pigs, although the macroscopic finding was confirmed microscopically.*

which was in poor condition, and finally became mired, was found dead on September 16, 1929. Post-mortem examination did not show evidence of tuberculosis. These animals, now reduced to thirty-three, seventeen vac-

cinated and sixteen controls, were slaughtered on October 31 and November 1, 1930, in the newly erected slaughter house at Ponoka Institute, and examined for the presence of tuberculous lesions. Guinea pigs were inoculated with glandular material and tissue was taken for microscopic examination.

At the time of slaughter these animals were over two years of age, and had been in contact with tuberculous animals, commencing nineteen months previously, for a period of more than six months. The vaccinated animals had been vaccinated five months previous to contact so that, at the termination of contact, over eleven months had passed since vaccination, and it was for this reason that contact was discontinued on November 1, 1929.

The results of this experiment perhaps indicate an increased resistance in the vaccinated animals—80% free from tuberculosis as compared to 60% in the controls. The lesions found were slight; glandular only in the three infected vaccinated animals, microscopic glandular lesions in one of the six infected controls, and organic lesions in only one. The chances of natural infection would appear not to have been as great as desired. These results are confirmed by microscopic examination of glandular material. As in the previous experiment the results of the macroscopic and microscopic examination of the tissues of the inoculated guinea pigs correspond to similar examination of the tissues of the bovines*. The detailed results of the examinations are given in Tables V, VI, VII, VIII, IX and X.

The experiment is interesting from the point of view of methods employed for the maintenance of quarantine and might be taken to illustrate the possibilities of tuberculous infection in young bovines so protected. We think also that it reflects creditably upon similar arrangements—used in our previously

TABLE IX

AUTOPSY FINDINGS (MACROSCOPIC AND MICROSCOPIC) ON SIXTEEN UNVACCINATED CONTROLS OF WHICH SIX SHOWED TUBERCULOUS LESIONS, EXPERIMENT 2, 1928–30

No. of animal	Nature and extent of post-mortem findings	Results of guinea pig inoculation
228	Portal gland shows, microscopically, scattered conglomerate tubercles not visible macroscopically.	Positive
231	One peri-bronchial lymph node shows small caseo-calcareous lesion. Lungs, small caseo-calcareous lesion.	Positive
235	Retro-pharyngeal lymph nodes show extensive caseous lesions.	Positive
237	One calcified mesenteric lymph node.	No test
239	Portal lymph node caseous.	No test, other glands negative
295	One mesenteric gland calcareous.	Positive
All others	Negative.	Negative

With respect to animals Nos. 237 and 239 the single tuberculosis gland found in each case was not inoculated into guinea pigs.

TABLE X

AUTOPSY FINDINGS (MACROSCOPIC AND MICROSCOPIC) ON SEVENTEEN VACCINATED ANIMALS, THREE OF WHICH SHOWED TUBERCULOUS LESIONS. EXPERIMENT 2, 1928-30

No. of animal	Nature and extent of post-mortem findings	Results of guinea-pig inoculation
242	One gastric lymph node showed a microscopic caseo-calcareous lesion. Macroscopically suspicious.	No test. All other glands negative
249	One of the retro-pharyngeal nodes caseo-calcareous.	Positive
305	One caseous mesenteric lymph node.	Positive
All others	Negative.	Negative

published experiments—for the exclusion from our experimental animals of tuberculous infection from outside sources. We are of the opinion that the duration of the experiment, which was over two years, strengthens any conclusions that seem justifiable.

Conclusions

1. B.C.G. vaccine is non-pathogenic for bovines.
2. Mouth vaccination with B.C.G. probably produces moderate resistance in bovines.
3. It would appear that new-born calves vaccinated by mouth are not capable of subsequently transmitting infection to non-vaccinated controls in close contact, and that vaccination is, therefore, no menace to non-protected animals.
4. B.C.G. does not produce bovine carriers of virulent tubercle bacilli.
5. As B.C.G. is a bovine strain, and has been shown to be non-pathogenic for young calves—the natural host—we think it may be assumed that, carried according to Calmette's method, this organism is non-virulent and will not dissociate or revert to type in the tissues.

Acknowledgments

The authors gratefully acknowledge the co-operation of the Minister of Health, and Department of Health of the Province of Alberta in the matter of assistance and accommodation, and the valuable assistance rendered by Dr. C. W. T. Haworth both in relation to the care of the animals, and in the practical aspects of the post-mortem examinations.

References

1. BUXTON, J. B. and GRIFFITH, A. S. Lancet, 220: 393-401. 1931.
2. CALMETTE, A. and GUERIN, C. Ann. inst. Pasteur, 38: 371-398. 1924.
3. RANKIN, A. C. Can. J. Research, 1: 48-85. 1929.

DISPERSION AND SELECTIVE ABSORPTION IN THE PROPAGATION OF ULTRASOUND IN LIQUIDS CONTAINED IN TUBES[1]

PART II

By G. S. Field[2] and R. W. Boyle[3]

Abstract

More experimental work has been done on the propagation of ultrasound along tubes filled with liquid. The mathematical theory developed by Field has been subjected to a quantitative verification, and it has been shown that the discontinuities in the velocity-frequency curve are due to a transference of energy at certain frequencies from the longitudinal to a radial vibration. It has also been shown that the theory satisfactorily accounts for the observed facts, the phase velocities, particle velocities and the absorption frequencies having the values which the theory requires.

Introduction

Following the experimental work of Boyle, Froman and Field (1), and the development of a formula by Field (3), to explain the experimental observations, it was decided to carry out a further research in order to subject the mathematical theory of the transference of energy to a radial vibration to a quantitative verification.

Accordingly, three separate experiments were undertaken, these being (1) a determination and comparison of observed with calculated phase velocities, over a range of frequencies involving two absorption bands; (2) a determination of the frequencies of five absorption bands in a tube of liquid and a comparison of the observed with the theoretical frequencies; and finally (3) a determination of radial particle velocities in a tube of liquid at a lower and a higher frequency than that of the first absorption band, and a comparison of these velocities with their calculated values from the theory.

Phase Velocities

To measure velocities of sound in tubes, the final method adopted in Part I was employed. That is to say, a rod-oscillator was fitted into each end of a glass tube, cavitation was produced in the liquid, and the distance between a number of nodes, as indicated by rising curtains of bubbles, was measured. Knowing the frequency of the electrical oscillator, the phase velocity of ultrasound in the liquid was then easily determined. In this way, velocities over a range of frequencies were obtained for naphtha in two different sizes of glass tubes. The results are plotted graphically in Figs. 1 and 2.

For the theoretical calculations, the following formulas were employed,

$$x\frac{J_1(x)}{J_0(x)} = \frac{\frac{-a}{h}}{\frac{E'}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}} \qquad (1)$$

**Reference 3, p. 142, formula 48.*

[1] *Manuscript received January 16, 1931.*
Contribution from the National Research Laboratories, Ottawa, Canada.

[2] *Junior Research Physicist, National Research Laboratories.*
[3] *Director, Division of Physics, National Research Laboratories.*

$$x' \frac{I_1(x')}{I_0(x')} = \frac{+\frac{a}{h}}{\frac{E'}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}} \qquad (2)$$

In the case of the first tube, the physical constants were: $a=0.97$; $h=0.13$; $\rho=0.74$; $\rho_1=2.6$; $E=6.03\times10^{11}$; $c_0=1.26\times10^5$.

$$E' = \frac{E}{1-\frac{5h}{6a}} = 6.78\times10^{11}.$$

Substituting the numerical values in Equation 1 gives,

$$x \frac{J_1(x)}{J_0(x)} = \frac{-1}{\frac{33.2\times10^8}{n^2} - 0.47} \qquad (3)$$

Each value of n ($n > n_0$, where n_0 = absorbing frequency) determines a value of $x\frac{J_1(x)}{J_0(x)}$, and hence of x. By tabulating a few values of $x\frac{J_1(x)}{J_0(x)}$ it is possible to fix x approximately, and trial and error will then readily give x to one or two decimal places. There is no particular point in determining x very exactly, as the calculations in any case involve physical constants whose values are known only approximately.

TABLE I

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 0.97 CM. IN RADIUS, FOR FREQUENCIES *above* ABSORPTION BAND

N in kilocycles/sec.	$X\frac{J_1(x)}{J_0(x)}$	X	$N_x=\frac{(xc)}{(2\pi a)}$	$N^2\times10^{-8}$	$N_x^2\times10^{-8}$	$(\sqrt{N^2-N_x^2})\times10^{-4}$	$C_1\times10^{-5}$
65	− 3.1	3.12	64.5	42.3	41.6	0.84	9.75
70	− 4.8	2.90	59.9	49	35.9	3.62	2.44
80	−20.1	2.53	52.3	64	27.4	6.02	1.67
90	+16.7	2.26	46.7	81	21.8	7.69	1.48
100	+ 7.1	2.10	43.4	100	18.8	9.01	1.40
110	+ 5.0	1.99	41.2	121	17.0	10.2	1.36
120	+ 4.2	1.93	39.9	144	15.9	11.3	1.34

TABLE II

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 0.97 CM. IN RADIUS, FOR FREQUENCIES *below* ABSORPTION BAND

N in kilocycles/sec.	$X^1\frac{J_1(x^1)}{J_0(x^1)}$	X^1	$N_x^1=\frac{(x^1C)}{(2\pi a)}$	$N^2\times10^{-8}$	$N_{x'}^2\times10^{-8}$	$(\sqrt{N^2\times N_{x'}^2})\times10^{-4}$	$C_1\times10^{-5}$
30	0.311	0.82	17	9	2.89	3.45	1.10
40	0.625	1.21	25	16	6.25	4.72	1.07
50	1.16	1.80	37.2	25	13.8	6.23	1.01
60	2.22	2.80	57.9	36	33.5	8.34	0.91
70	4.77	5.2	108	49	116.6	12.9	0.68
80	20	2.1	434	64	1884	44.1	0.23
84	∞	∞	∞	70.6	∞	∞	0

*Reference 3, p. 143, formula 59.

The results of calculations of phase velocity for a number of frequencies are shown in Table I and plotted in Fig. 1.

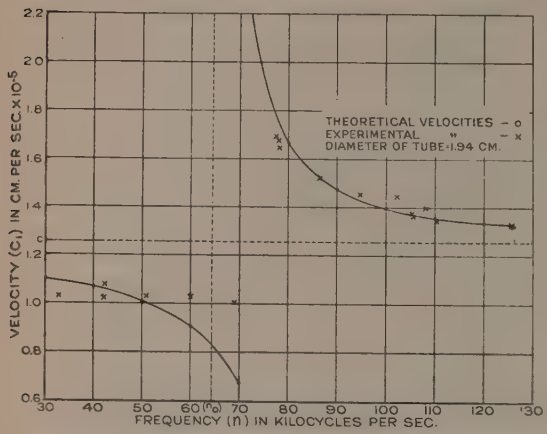

THEORETICAL VELOCITIES — o
EXPERIMENTAL " — x
DIAMETER OF TUBE-1.94 CM.
VELOCITY (C₀) IN CM. PER SEC. X 10⁻⁵
FREQUENCY (n) IN KILOCYCLES PER SEC.

FIG. 1. *Comparison of theoretical and experimental phase velocities for naphtha in a glass tube, 1.94 cm. diameter and 1.2 mm. wall thickness.*

For low frequencies it is necessary to use Equation 2. When the numerical values are substituted, an equation similar to Equation 3 is obtained. Values of x' are determined as above. Using these values, phase velocities were calculated for frequencies below the first absorption band. The results are shown in Table II and plotted in Fig. I.

For the second tube, $a = 1.55$; $h = 0.14$; $c_0 = 1.21 \times 10^5$ (different sample of lighting naphtha); other constants as before.

In this case we have for high frequencies,

$$x \frac{J_1(x)}{J_0(x)} = \frac{-1}{\frac{8.76 \times 10^8}{n^2} - 0.316} \tag{4}$$

And for low frequencies,

$$x' \frac{I_1(x')}{I_0(x')} = \frac{1}{\frac{8.76 \times 10^8}{n^2} - 0.316} \tag{5}$$

Calculations of velocity were made from these two formulas and are shown in Tables III and IV. The theoretical curve resulting therefrom is shown in Fig. 2.

In order to show the effect of radial resonance for modes other than the fundamental, a calculation was made of a few velocities at frequencies just above that of the first overtone on the assumption that resonance was occurring at this frequency.

Mathematically, this means simply taking the second solutions for x, and carrying through the calculations therewith. Table V shows the results of these computations and in Fig. 2 the theoretical velocities are plotted.

Remarks

The agreement between calculated and observed values of velocity on the high frequency side of the absorption band is seen to be very good. On the low frequency side the theoretical velocities are too low in the immediate vicinity of the absorbing frequency. This was expected, however, and an explanation was given in the theoretical paper (3), where it was suggested that if viscosity were included in the analysis, better agreement would most likely be obtained.

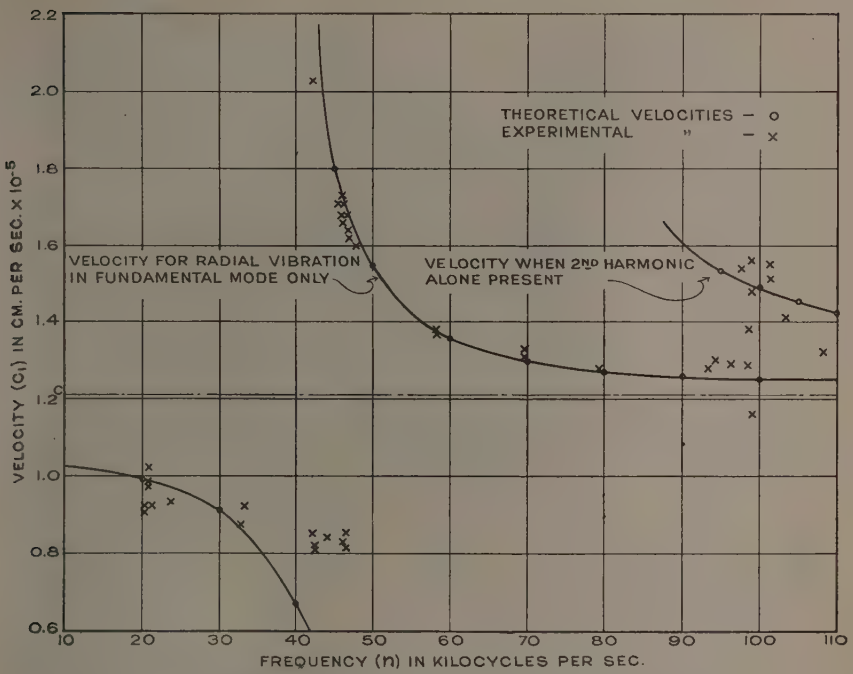


FIG. 2. *Comparison of theoretical and experimental phase velocities for naphtha in a glass tube, 3.1 cm. diameter and 1.4 mm. wall thickness.*

TABLE III

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 1.55 CM. IN RADIUS, FOR FREQUENCIES *above* FIRST ABSORPTION BAND

N in kilocycles/sec.	$X\frac{J_1(x)}{J_2(x)}$	X	$N_x=\left\{\frac{CX}{2\pi a}\right\}$	$N^2\times10^{-8}$	$N^2\times10^{-8}$	$\left(\sqrt{N^2-N_x^2}\right)\times10^{-4}$	$C_1\times10^{-5}$
100	4.38	1.94	24.1	100	5.81	9.71	1.25
90	4.81	1.97	24.5	81	6.00	8.66	1.26
80	5.58	2.03	25.2	64	6.35	7.59	1.28
70	7.30	2.10	26.1	49	6.81	6.50	1.30
60	13.7	2.23	27.7	36	7.67	5.32	1.36
50	−29.4	2.50	31.1	25	9.67	3.91	1.55
45	−8.62	2.70	33.5	20.3	11.22	3.02	1.80
40	−4.33	2.97	36.9	16	13.62	1.55	3.12

At high frequencies, where first overtone resonance is beginning to occur, the phase velocities become indefinite. The theoretical curves suggest that either one or both of two different velocities may occur, and the experimental readings indicate that the actual velocity has a definite value somewhere between the two, except at about 99 kilocycles, where the velocity may be anything between the two. This seems to be a sort of change-over point,

TABLE IV

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 1.55 CM. RADIUS, FOR FREQUENCIES *below* FIRST ABSORPTION BAND

N in kilocycles/sec.	$X\frac{I_1(x^1)}{I_o(x^1)}$	X^1	$N_{x'}=\left(\frac{CX^1}{2\pi a}\right)$	$N^2\times10^{-8}$	$N_{x'}^2\times10^{-8}$	$(\sqrt{N^2+N_{x'}^2})\cdot\times10^{-4}$	$C_1\times10^{-5}$
20	0.534	1.11	13.8	4	1.90	2.43	0.99
30	1.54	2.14	26.6	9	7.08	4.01	0.91
40	4.32	4.88	60.6	16	36.7	7.26	0.67
50	29.4	30	373	25	1390	37.6	0.16

TABLE V

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 1.55 CM. IN RADIUS FOR FREQUENCIES *above second* ABSORPTION BAND

N in kilocycles/sec.	$X\frac{J_1(X)}{J_o(x)}$	X	$N_x=\left(\frac{CX}{2\pi a}\right)$	$N^2\times10^{-8}$	$N_x^2\times10^{-8}$	$(\sqrt{N^2-N_x^2})\cdot\times10^{-4}$	$C_1\times10^{-5}$
120	3.92	4.59	57	144	32.5	10.6	1.37
110	4.09	4.61	57.3	121	32.8	9.39	1.42
105	4.22	4.63	57.5	110	33.1	8.77	1.45
100	4.38	4.65	57.8	100	33.4	8.16	1.49
95	4.57	4.67	58.0	90.3	33.6	7.53	1.53

where the velocity goes from one type of vibration to the other. This corresponds to the frequency 45 kilocycles, where two distinct groups of velocities were obtained, one group with an average high value and the other with a low.

Absorption of Sound Energy in the Tube of Liquid

To measure the absorption of energy in a tube containing liquid, apparatus was set up as shown in Fig. 3. *A* and *B* are two galvanized iron tanks, with an arrangement at one end for admitting a glass tube (Tank *B*) and a piston oscillator (Tank *A*) and for making water-tight connections therewith. T_c and T_l are torsion pendula with their accompanying torsion heads and telescopes, similar to apparatus previously used in ultrasonic energy measurements. The pendula used in this research were the single "air" vane type (2).

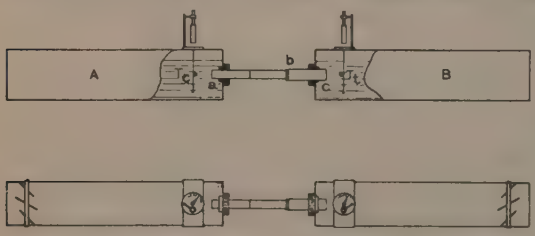


FIG. 3. *Apparatus used for experimentally determining the proportion of energy transmitted along a tube at different frequencies.*

To measure the absorption in the tube of liquid, the most direct way would be to measure the energy of the sound at *b* and *c*. The difference between the two energy measurements would then represent the amount absorbed by the

cylinder of liquid between b and c. It is, however, almost impossible to measure energies reliably inside a tube, such as at b, so that it was necessary to employ an indirect method which would give equivalent results. In this case, the difficulty was overcome by taking readings at a. In doing this it was tacitly assumed that the amplitude of vibration of the oscillator at a was equal to the amplitude at b. This was probably not entirely true, but since each end was vibrating in the same liquid and each was clamped with a rubber washer (one at the tank and one at the glass tube), the difference should not have been great. The experimental results appeared to justify the assumption made.

It was also assumed that the energy field about the end of the rod at a varied with frequency in about the same manner as the field about the end of the tube at c, because at the frequencies used no interference phenomena could intervene, such as the formation of a beam of ultrasound from the ends of the oscillator or tube, or the production of any regions of maximum or minimum energy intensity along the axis. Hence deceptive readings of the measuring pendula could not occur.

Let P_c = deflection of pendulum T_c, and P_t = deflection of pendulum T_t.

Then $K\frac{P_t}{P_c}$ = proportion of energy transmitted through tube, where K = a constant, which would equal unity if the energy intensities at a and b were exactly similar, and if the two pendula were exactly the same.

By plotting $\frac{P_t}{P_c}$ against frequency, therefore, we obtain a curve of relative transmission at the different frequencies.

In order to obtain reasonable readings with the torsion pendula, it was necessary to operate the rod oscillator at a resonance point. This meant that any slight variation in frequency caused a large change in radiated energy and hence a large variation in pendula readings. After a number of vain attempts to keep the frequency sufficiently constant to obviate the difficulty, it was found that fairly consistent results could be obtained by watching each pendulum in turn and taking the maximum readings. Three or four such readings were taken in each case, and the average noted.

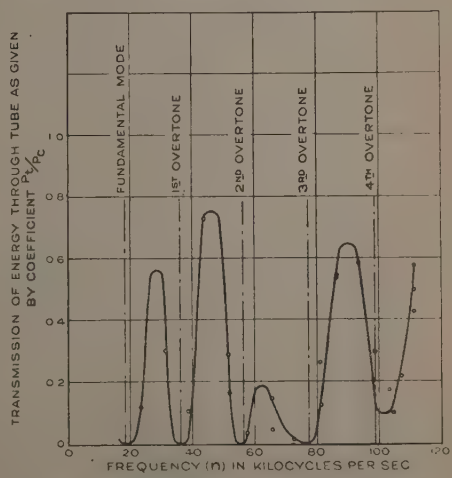


FIG. 4. *Curve showing the absorption bands occurring for energy transmitted through water contained in a glass tube.*

Since several different suspensions were used, on account of breakages, it was necessary to correlate the different sets of readings to allow for different

values of K. This was done by comparing deflections taken at the same frequency, but with the different suspensions. Care was taken that the frequency used for correlating readings was one which corresponded to large pendula deflections, since these readings would most likely have the smallest percentage error and for these frequencies the absorption had minimum values. The experimental results are given in Table VI and plotted in Fig. 4.

In order to compare the observations with the theory, the frequencies of radial resonance were calculated using the formulas,

$$\frac{J_1(x)}{J_0(x)} = \frac{-xac^2\rho}{h(E'-x^2c^2\rho_1)} \quad * \tag{6}$$

$$n = \frac{cx}{2\pi a} \quad ** \tag{7}$$

In this case, $a=3.38$, $h=0.18$, $c=1.52\times10^5$, $E'=6.78\times10^{11}$, $\rho_1=2.7$, $\rho=1.0$.

Hence,

$$\frac{J_1(x)}{J_0(x)} = \frac{-x}{1.6-0.144x^2} \tag{8}$$

$$n = \frac{cx}{2\pi a} \tag{9}$$

From these equations the frequencies of radial resonance were calculated to be as in Table VII, and indicated on the absorption curve by vertical lines.

TABLE VI

EXPERIMENTAL DETERMINATION OF ABSORPTION AND TRANSMISSION OF SOUND ALONG A TUBE CONTAINING WATER

Frequencies in kilocycles	P_c deflection in deg.	P_t deflection in deg.	$\frac{P_t}{P_c}$	Correlated $\frac{P_t}{P_c}$
65.6	105	5	0.05	0.05
72.5	99	0	0.00	0.00
81.0	53	14	0.26	0.26
86.8	62	34	0.55	0.55
93.7	117	69	0.59	0.59*
51.6	27	4½	0.17	0.17
65.5	89	13	0.15	0.15
72.5	139	3	0.02	0.02
81.3	64	8	0.13	0.13
86.2	31	24	0.77	0.53
93.6	44	38	0.86	0.59*
99.0	39	17	0.44	0.30†
58.3	61	7	0.11	0.08
57.3	47	3	0.06	0.04
51.6	24	10	0.42	0.29
43.5	14	15	1.07	0.73
38.3	166	26	0.16	0.11
31.0	50	22	0.44	0.30
23.1	45	8	0.18	0.12
99.0	73	28	0.38	0.30†
97.8	30	15	0.50	0.39
103	30	7	0.23	0.18
104.7	40	5	0.13	0.10
106.6	37	10	0.27	0.21
111.2	110	59	0.54	0.43
	66	49	0.74	0.58
111.7	114	73	0.64	0.51

*Readings correlating groups 1 and 2. †Readings correlating groups 2 and 3.

*Reference 3, p. 138, formula 22.

**Reference 3, p. 138, formula 23.

TABLE VII

FREQUENCIES OF RADIAL RESONANCE IN TUBE OF WATER, AS CALCULATED FROM THEORY

Mode of vibration	Fundamental	1st overtone	2nd overtone	3rd overtone	4th overtone
Frequency, cycles/sec.	18,800	36,600	56,400	77,400	99,000

Radial Particle Velocities

In attempting to measure radial particle velocities in the tube, it was hoped to show three things: first, that a radial vibration was actually occurring at certain frequencies in the liquid even though presumably only a longitudinal wave was started at one end; second, that the type of vibration above the frequency of the first absorption band was different from the type at a frequency below it; and third, that the particle velocities were substantially as indicated by the theory.

To measure these particle velocities, a new experiment was devised making use of the theory of the Rayleigh disk. A tube of water was mounted vertically and set into vibration by a rod oscillator fitted with a rubber gasket to the lower end. A Rayleigh disk (0.8 cm. in diameter) was made of copper sheet, 0.47 mm. thick, and suspended in the liquid by a single silk fibre from a suitable torsion head. The reason for using the silk fibre was that the turning moment produced by the movement of the particles of liquid was very slight, and other suspensions (*e.g.*, a very thin gold wire) which were tried were found to be too stiff to give a good angle of twist.

The disk was viewed with a telescope and set approximately at 45° to a radius vector. The oscillator was started and the tendency of the disk to rotate, caused by the action upon it of the particles of liquid, was compensated by twisting the fibre suspension at the torsion head.

The twisting moment on the disk caused by the moving liquid is given by the relation,

$$M = K\dot{\xi}^2 \sin 2\varphi \quad * \qquad (10)$$

Where M = twisting moment, K = a constant, $\dot{\xi}$ = particle velocity, φ = angle between the normal to the disk and the direction of the undisturbed stream.

Since the disk was kept in a constant position by twisting the fibre, φ was constant, and therefore, $M = K'\dot{\xi}^2$. Now the counter-torque of the fibre produces a moment, $M' = c\Theta$, where Θ = angle of rotation of torsion head.

Since $M = M'$, $K'\dot{\xi}^2 = c\Theta$.

Or $$\dot{\xi}^2 = c'\Theta.$$

This is the equation relating the particle velocity to the angle of twist of the torsion head.

The first absorption band for the liquid and tube used occurred at 18,000 cycles/sec. Readings were therefore taken at about 14,000 cycles and about 23,000 cycles, corresponding to convenient frequencies above and below the absorption frequency. The results are given in Tables VIII and IX.

**Reference 4, vol. 2, p. 44.*

In order to compare the experimental observations with the theoretical values, use was made of the relations,

$$\dot{\xi}_{a<c} = -\alpha I_1(\alpha r) A e^{iw\left(t-\frac{Z}{c_1}\right)} \quad *$$

$$\dot{\xi}_{a>c} = k J_1(kr) A e^{iw\left(t-\frac{Z}{c_1}\right)} \quad **$$

The average values of these velocities are,

$$\dot{\xi}_{a<c} = a_1 I_1(\alpha r)$$

$$\dot{\xi}_{a>c} = a_2 J_1(kr)$$

Where a_1 and a_2 are constants. By putting a_1 and a_2 equal to unity and finding α and k, it is possible to calculate values proportional to the particle velocities.

To find α and k, we use Equations 1 and 2, in which $x = ka$, and $x' = \alpha a$.

In this experiment, water in a glass tube was employed and the physical constants are the same as were used in the calculations for the absorption frequencies.

To find αa, we use $n = 13{,}900$, and for ka, $n = 23{,}200$.

Solving Equations 1 and 2, we obtain, $x' = \alpha a = 3.42$, or $a = 3.42/3.38 = 1.01$: $x = ka = 2.44$ or $k = 2.44/3.38 = 0.722$.

Hence, $\dot{\xi}_{a<c} = I_1(1.01r)$, $\dot{\xi}_{a>c} = J_1(0.72r)$.

From these equations theoretical values proportional to particle velocities were calculated and are shown in Tables VIII and IX. Values of the squares of particle velocities are also shown as they are proportional to the turning moments produced on the Rayleigh disk and hence to the angles of twist (Θ) of the torsion head. In the last columns of Tables VIII and IX are values related to $[I_1(\alpha r)]^2$ and $[J_1(kr)]^2$ which have been calculated from the observed values of Θ. The first value in each group was used to determine the constant, K, relating Θ to particle velocities, while the rest of the values represent experimental observations.

TABLE VIII

PARTICLE VELOCITIES IN TUBE OF WATER, *before* FIRST ABSORPTION BAND

r	αr	$I_1(\alpha r)$	$\{I_1(\alpha r)\}^2$	Θ, degrees	$K\Theta$
2.6	2.63	2.83	8.01	1000	8.01
2.0	2.02	1.62	2.62	330	2.64
2.3	2.33	2.16	4.67	1320	4.67
1.6	1.62	1.11	1.23	190	0.67
1.0	1.01	0.57	0.32	—	—
0.5	0.51	0.26	0.07	—	—

The theoretical curves have been plotted in Fig. 5, and the experimental points have been marked thereon. It can be seen that the agreement is satisfactory.

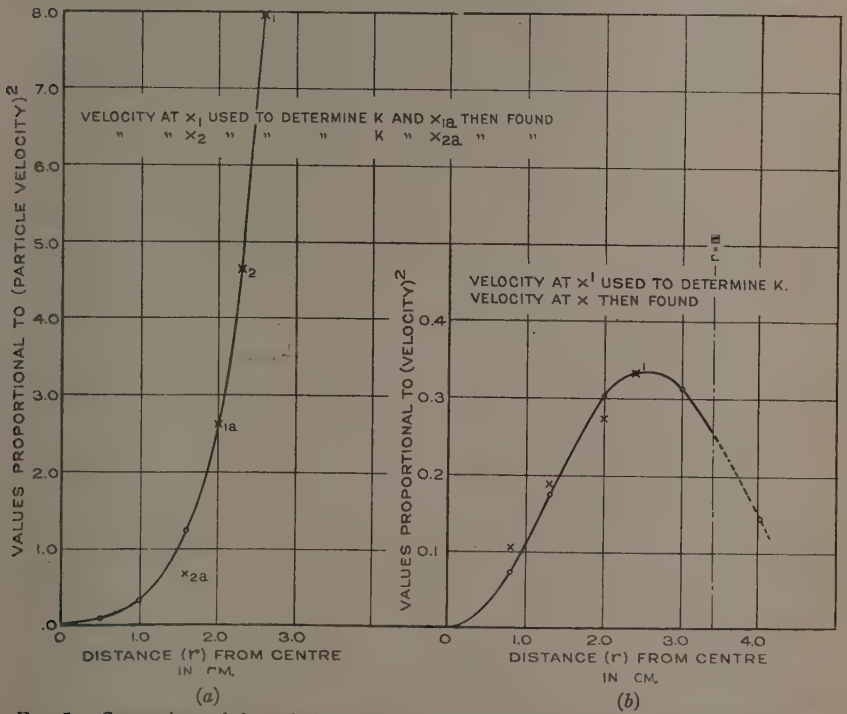
**Reference 3, p. 140, formulas 38 and 53.*

***Reference 3, p. 141, formula 41.*

TABLE IX

PARTICLES VELOCITIES IN TUBE OF WATER, *after* FIRST ABSORPTION BAND

r	kr	$J_1(kr)$	$\{J_1(kr)\}^2$	Θ, degrees	$K\Theta$
2.4	1.73	0.579	0.335	9×360	0.335
2.0	1.45	0.550	0.303	16×360	0.275
1.3	0.94	0.420	0.176	23×360	0.191
0.8	0.58	0.278	0.077	28×360	0.108
3.0	2.16	0.561	0.315	—	—
4.0	2.89	0.379	0.144	—	—


VELOCITY AT x1 USED TO DETERMINE K AND x1a THEN FOUND
" " x2 " " " K " x2a " "
VALUES PROPORTIONAL TO (PARTICLE VELOCITY)²
DISTANCE (r) FROM CENTRE IN CM.
VELOCITY AT x' USED TO DETERMINE K.
VELOCITY AT x THEN FOUND
VALUES PROPORTIONAL TO (VELOCITY)²
DISTANCE (r) FROM CENTRE IN CM.

(*a*) (*b*)

FIG. 5. *Comparison of theoretical and experimental radial particle velocities for sound transmitted through water contained in a glass tube; (a), at a lower frequency than that of the fundamental radial vibration, (b), at a higher frequency than that of the fundamental.*

Conclusions

As a result of the foregoing experiments, it has been established that the discontinuities in the velocity-frequency curve for sound transmitted in liquids contained in cylindrical tubes are due to selective absorption of the longitudinal vibration taking place at the resonant frequencies of the radial vibration. In addition, it has been shown that the theory satisfactorily accounts for the observed facts in connection with the transmission of sound along tubes, the

phase velocities, particle velocities and the absorption frequencies having the values which the theory requires.

References

1. Boyle, R. W., Froman, D. K. and Field, G. S. Can. J. Research, 6: 102-118. 1932.
2. Boyle, R. W. and Lehman, J. F. Can. J. Research, 3: 491-509. 1930.
3. Field, G. S. Can. J. Research, 5: 131-148. 1931.
4. Raleigh, Lord. Theory of sound. Macmillan. 1926.

STUDIES OF POLYMERS AND POLYMERIZATION

IV. OBSERVATIONS ON THE POLYMERIZATION OF ISOPRENE AND 2, 3-DIMETHYLBUTADIENE-1, 3[1]

By George Stafford Whitby[2] and Robert Nelson Crozier[3]

Abstract

The polymerization of isoprene and dimethylbutadiene at temperatures ranging from 10° to 145° C. has been studied with reference to the nature of the dimeric products and the influence of the temperature and period of heating on the extent of polymerization, the ratio of oil to rubber and the molecular weight of the rubber and viscosity of its sols. There was no evidence of the formation from either diene of an open chain dimer, polymerizable to rubber, such as the "β-myrcene" of Ostromislenski. The ratio of oil:rubber from a given diene is approximately constant in different periods of polymerization at a given temperature. The ratio increases with rise of temperature. Isoprene gives far more oily by-product than dimethylbutadiene. As polymerization progresses, the molecular weight of the rubber and the viscosity of its sols rises. The higher the temperature of polymerization, the lower is the molecular weight ultimately attained. The polymerization is catalyzed by air. The viscosity of the sols of the synthetic rubbers is low and falls on keeping owing to oxidation.

Ostromislenski (27, 28) has described the preparation from isoprene, by heating, of a dimer, "β-myrcene", for which he suggested the constitution CH_2:CMe.CH_2.CH_2.CH:CMe.CH:CH_2, and which, he stated, is polymerizable to caoutchouc. If these observations can be substantiated they are clearly of first-rate significance in regard to the mechanism of the polymerization of isoprene to caoutchouc and in regard to the constitution of the latter, and the establishment of the constitution of "β-myrcene" is a matter of importance. The present work was undertaken first with the object of isolating and studying "β-myrcene" or other simple intermediate products in the polymerization of isoprene to caoutchouc. All attempts to isolate "β-myrcene" were, however, unsuccessful. The procedures described by Ostromislenski were carefully followed, but no evidence of the formation of the substance was obtained (*vide infra*). The only oily products, described later, which could be isolated when isoprene and dimethylbutadiene were polymerized by heat were cyclic dimers such as have been described by previous authors. These compounds are not further polymerizable to caoutchoucs; they are not intermediate steps in the passage from the dienes to caoutchoucs, and have no special significance in regard to the mechanism of the polymeric process by which caoutchoucs are formed.

Following the examination of the oily polymers, experiments were carried out on the influence of temperature and time of heating on the polymerization of isoprene and 2, 3-dimethylbutadiene-1, 3, with special reference to the yields of oily and rubber-like polymers respectively and with reference to the molecular weight of the rubber and the viscosity of its sols. The most important data are summarized in Table I.

[1] *Manuscript received January 30, 1932.*

Contribution from the Chemistry Department, McGill University, Montreal, based on a thesis by R. N. Crozier, April, 1928.

[2] *Director, Division of Chemistry, National Research Council, Ottawa.*

[3] *Holder, at the time, of a studentship under the National Research Council of Canada.*

TABLE I

POLYMERIZATION OF ISOPRENE AND 2, 3-DIMETHYLBUTADIENE-1, 3

Time, hr.	Isoprene				2, 3-Dimethylbutadiene-1, 3			
	Oil %	Rubber, %	Mol. wt.	Rel. visc.*	Oil, %	Rubber, %	Mol. wt.	Rel. visc.*
At 85° C.								
97.5	9.07	17.05	4589	4.62				
101	7.9	16.25			0.9	10.5	2318	3.69
154					1.5	13.8	3345	3.83
250					2.7	19.6	3524	4.55
900		35.3	5715	8.20		49.7	3483	9.21
At 145° C.								
5.25	38.2	10.03	3291	3.14				
12.5	54.7	15.56	3936	4.69	11.1	15.6	2138	2.3
At 45° C.								
2628					Trace	19.25	2106	2.76
At 10° C.								
1 year	0.005	0.01		6.13**				
At room temp.								
80 days					None	3.45	1926	1.99
4½ years	1.0	16.6	2312	13.4†				

*Relative viscosity at 30.1° C. of sol containing approx. 0.68 gm./20 cc. benzene.
**Relative viscosity at 30.1° C. of sol containing approx. 0.492 gm./20 cc. benzene.
†First fraction (see page 216).

The results at 145° C. are in good accord with those obtained by Lebedev (20, p. 1313) at 150° C. At this temperature he found the total polymerizate from isoprene to be 53% after five hours and 79% after 15 hr., while from dimethylbutadiene the total polymerizate after 15.5 hr. was 39.7%. He found that when the polymerization of isoprene at 150° C. was complete, the product contained only 10% of rubber.

As an examination of Table I shows, the rate of polymerization of isoprene when heated is greater than that of dimethylbutadiene. This is perhaps somewhat unexpected, as the authors have observed, in accord with most previous workers, that at room temperature dimethylbutadiene undergoes polymerization far more readily than isoprene. Samples of the former were generally observed to undergo complete polymerization to typical, white, cauliflower masses when kept for a year or two (Kondakov (12), who first observed the spontaneous polymerization of dimethylbutadiene, found it to be complete in one year), whereas a sample of isoprene kept for 4½ years showed no separation of solid polymer and only about 16% polymerization to caoutchouc.

Even when considerably diluted, samples of dimethylbutadiene were observed by Macallum and Whitby (23) to undergo on keeping conversion to the solid polymer after two or three years. Thus, *e.g.*, a fraction prepared from pinacone, boiling at 90 to 100° C. and containing a large proportion of pinacoline, showed on keeping considerable separation of polymer.

Heat polymerization leads to the formation of a much larger proportion of oily by-products in the case of isoprene than in that of dimethylbutadiene. Increase in the temperature applied raises the proportion of oil to caoutchouc in both cases. In the experiments at 145° C. the oil formed from isoprene is more than three times the amount of caoutchouc formed. This makes it clear that the temperatures specified for the polymerization of isoprene to rubber in certain patents (*e.g.*, 4) are too high.

Some uncertainty attaches to the figures given for the molecular weight of the polymeric products, owing to the fact that the results of cryoscopic determinations were affected by the concentration of the solutions used. The results may, however, be considered as approximately correct, and, regarded broadly, show that, on heating either of the dienes at a given temperature, the molecular weight of the rubber produced increases as the percentage of the diene which has undergone polymerization rises. Further, the higher the temperature applied to produce polymerization, the lower is the molecular magnitude of the rubber produced after similar amounts have been formed. And, again, as polymerization at a given temperature progresses, the viscosity of sols of the product rises. These generalizations are all in accord with those established by Whitby and Katz (37, 38) in a study of the polymerization of indene, namely, that, as polymerization by heat progresses at a given temperature, the state of polymerization rises, and the higher the temperature applied, the lower is the state of polymerization which is attainable in the ultimate product.

Since the polymerization of dimethylbutadiene at 85°C. is accompanied by the formation of only small amounts of oily, dimeric by-products, it was possible to follow the course of the polymerization by following the change of the refractive index. The results obtained in this connection are illustrated by the curve in Fig. 1. The refractive index changed rapidly at first, and then the rate of change slowed up considerably. This is in accord with the prior experiments (the results of which are given in Table I), in which

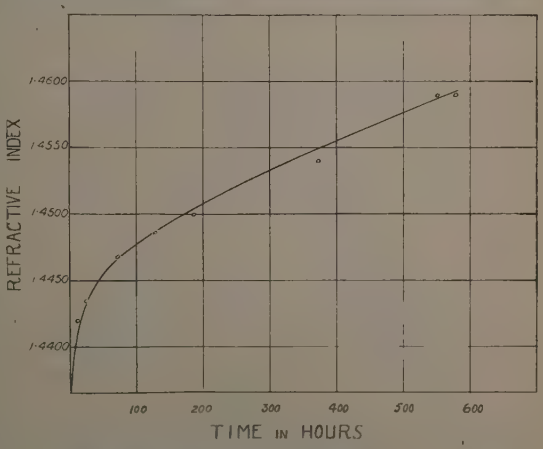


FIG. 1. *Change of refractive index of dimethylbutadiene with heating at 85°C.*

the polymerization products were isolated and weighed; polymerization proceeds rapidly up to a certain point and then slows down. During the first 24 hr. heating at 85° C. the refractive index of dimethylbutadiene rose from 1.4367 to 1.4435, whereas after heating for 15 days and 12 hr. it rose only to 1.4540.

The rubber obtained by the polymerization of dimethylbutadiene at 85° C. dispersed in a few hours when placed in benzene, whereas that obtained by its polymerization at room temperature, which is presumably of much higher molecular weight, only swelled in benzene and had not dispersed after two months' standing.

By fractional precipitation it was found possible to separate dimethylbutadiene caoutchouc into fractions which gave sols possessing, at the same concentration, different viscosities. This shows that the polymeric products consist of a mixture of polymers representing different degrees of polymerization. A similar heterogeneity has previously been shown in the case of polymerizates from indene (37, 38), vinyl acetate (40) and styrene (31, 39).

Measurements made of the iodine absorption of dimethylbutadiene caoutchouc point to the presence, as in natural caoutchouc, of one double bond for each unit of the diene which has gone to form the polymer. This is in agreement with the molecular refraction as determined by Macallum and Whitby (22).

Sols of the caoutchoucs obtained by the heat polymerization of both isoprene and dimethylbutadiene were observed to suffer a marked decrease in viscosity on keeping. For instance the time of flow of a sol of dimethylbutadiene caoutchouc which was originally 155.4 sec. had fallen after only 25 days in the dark to 81.6 sec., and that of a sol of isoprene caoutchouc from a time of flow of 221.2 sec. 7 days after its preparation to 125.2 sec. after 46 days. That the fall in viscosity is due to the gradual oxidation of the synthetic rubber is made apparent by the further observation that the addition of an antioxidant, dimethylaniline, prevented a fall in the viscosity on keeping. Fig. 2 shows the fall in viscosity of sols of methyl caoutchouc with time. The fall resulted from a permanent change in the caoutchouc, since on recovering the polymer from a sol which had been kept it was found to have been

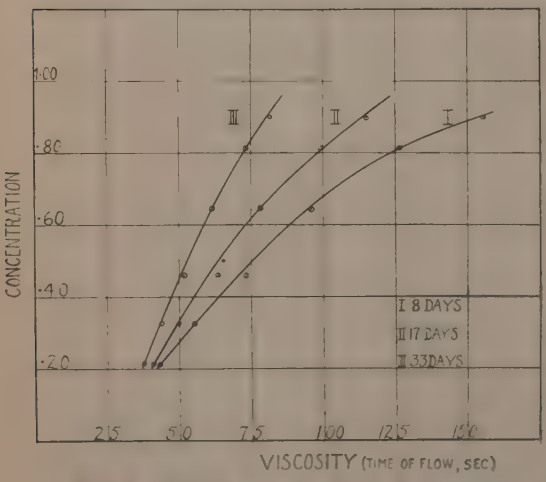


FIG. 2. *Fall in viscosity of sols of dimethylbutadiene caoutchouc on keeping. Time of flow measured after keeping sols for 8, 17 and 33 days.*

converted into a sticky product different from the originally tough, elastic rubber.

Various inorganic halides were found to bring about the rapid catalytic polymerization of isoprene and dimethylbutadiene in the cold. The most vigorous catalyst observed appeared to be antimony pentachloride. Other powerful catalysts were stannic chloride, antimony trichloride and aluminium chloride. Less effective were ferric chloride and bromide, thorium bromide, boron trichloride and tribromide and dichloracetic acid. These yielded a small quantity of polymer after standing two days. The polymers produced by the various halides were in all cases white or pale-colored substances, inelastic and quite unlike rubber. Apparently they are isomers of rubber. It is interesting to note that the halides converted natural rubber into similar products. On adding them to a solution of rubber in benzene highly colored complex products were formed first, and these, when alcohol was added, yielded white or pale-yellow powders, mostly soluble in benzene to only a slight extent. A similar observation in the case of the action of stannic chloride on rubber has been made by Bruson, Sebrell and Calvert (6).

Dimers

By heating isoprene at 280-290° C. for six hours, G. Bouchardat (5) obtained among other products a terpenic fraction which boiled chiefly at 176-181° C., and had d° 0.866, d^{21} 0.853. It was designated terpilene. Tilden (32) heated isoprene at 250-280° C. for 12 hr. and found on distillation that about half the material came over between 170 and 180° C. Wallach (34) identified dipentene in oils such as those just mentioned. He heated isoprene for some hours at 250-270° C. and obtained, together with high boiling polymerization products, a hydrocarbon, boiling at 180° C., which yielded a tetrabromide identical with dipentene tetrabromide. Wallach (34) also identified dipentene in the oil obtained by the destructive distillation of rubber, and it came to be generally considered that dipentene constitutes the major part of the terpene fraction in the oil obtained by the dry distillation of rubber. Harries (8) however, states that dipentene constitutes at most one-third of the "dipentene fraction" boiling at 150-200° C. obtained from rubber.

Harries further states that on heating isoprene at 300° C. he found only a little dipentene to be formed, the main product being another terpene boiling at 64-66° C./12 mm.

Lebedev (18, 19, 21) subjected isoprene to a lower temperature than the temperatures mentioned above, namely, 150° C. He obtained besides dipentene another dimer which he considered to be 1,3-dimethyl-3-ethenyl-6-cyclohexene. The properties of the two fractions he obtained were as shown in Table II.

In the experiments recorded in the present paper Lebedev's dimer was not encountered. Harries (9) in a later paper than that mentioned above reports on the terpene fraction of freshly polymerized isoprene, without however specifying exactly the conditions under which polymerization was carried out, although apparently they involved heating isoprene with acetic acid at a tem-

TABLE II

PROPERTIES OF FRACTIONS OBTAINED BY LEBEDEV ON HEATING ISOPRENE TO 150° C.

Fraction	B.p. at 760 mm.	B.p. at 9 mm.	d_0^{20}	n_D^{20}
No. 1 (Lebedev's dimer)	160-161° C.	44°	0.8331	1.46581
No. 2 (Dipentene)	174-175° C.	58°	0.8454	1.47428

perature of about 100° C. The main quantity he found to boil at 63-65° C./14 mm. and to show d_{18}^{18} 0.8451, n_D^{18} 1.4708. It gave only a little dipentene tetrabromide. Harries considered that it probably consisted for the greater part of an open-chain dimer of isoprene of the same formula as that later assigned to "β-myrcene" by Ostromislenski (*infra*). The product was not investigated further. Aschan also has reported on a dimeric product from isoprene, which he regards as quite distinct from dipentene, but which he classifies as a cyclic compound of the sylvestrene series, and to which he assigns the name diprene. After being allowed to stand at ordinary temperature for 10 years, he found, that 250 gm. of isoprene yielded (2) 7 gm. of dimeric material, the major fraction of which had the properties recorded below and yielded a dihydrochloride which melted at 51.5-52° C. and depressed the melting point of dipentene dihydrochloride when mixed with the latter (m.p. of dipentene hydrochloride: 48-50° C.). Aschan (1, 3) also examined somewhat larger quantities of dimeric material obtained by the polymerization of isoprene "at temperatures below 100° C." (it is not stated whether a catalyst was used in the polymerization), and concluded that this too consisted mainly of diprene. The physical properties of the main fraction obtained by distillation under ordinary and under reduced pressure are shown below (*b* and *c*). A sample of "diprene" isolated in the present investigation from isoprene which had been allowed to stand at room temperature for $4\frac{1}{2}$ years was proved to contain dipentene by the preparation from it of dipentene tetrabromide.

TABLE III

PROPERTIES OF ASCHAN'S DIPRENE

Polymerization conditions	Boiling point, °C.	d_4^{20}	n_D
(a) By cold polymerization of isoprene	171-172/769 mm.	0.8535	
(b) By polymerization of isoprene below 100°	171.5-173/752 mm.	0.8481	1.46960*(23.4° C.)
(c) By polymerization of isoprene below 100°	68.5-69/16 mm.	0.8476	1.46946(23.2° C.)

**The figure given in the reference, viz. 1.49660, is apparently due to a clerical error.*

With the possible exception of the dimeric products mentioned by Harries (9) all the dimers just described as being obtained from isoprene are clearly cyclic compounds. The observed refractive index for both Lebedev's and Aschan's

products agree reasonably well with the calculated value for $C_{10}H_{16}\sqrt{_2}$. An entirely different dimer, *viz.*, "β-myrcene", has however been described by Ostromislenski (27, 28). It is stated that this hydrocarbon is formed when isoprene, either alone or in the presence of a catalyst, is allowed to stand at ordinary temperature or is heated to not above 150° C. (27). It is stated to be the chief product when isoprene is heated to 80-90° C. for 3-5 days (28). It is also stated to be an open chain compound containing three double bonds of which two form a conjugated system; to be polymerizable quantitatively to caoutchouc; to yield, like other conjugated hydrocarbons, an amorphous solid with an aqueous solution of sulphur dioxide. It is distinguished from the dimers of isoprene described by all other authors in possessing a much higher refractive index than they. The present authors find, it may be mentioned here, that the value recorded by Ostromislenski is too high even for the assumed constitution.

"β-myrcene"

B.p. 63.5°C./20 mm. n_D^{26}, 1.53681. d_4^{20}, 0.8472. MR_D: Found, 50.01; calcd., 47.8 (for $C_{10}H_{16}\sqrt{_3}$ +0.98 for exaltation).

As early as 1900-1902 Kondakov (14) had suggested "that a transient stage in the polymerization of these hydrocarbon conjugated dienes consists of dimeric, open chain isoprenes, containing several double bonds in the molecule." But prior to Ostromislenski's work on β-myrcene there was no clear record of the isolation of such an open chain dimer. In the present investigation attention was particularly directed to β-myrcene when studying the oily dimers from isoprene. The conditions under which the polymerization of isoprene were brought about were all conditions which Ostromislenski has indicated as suitable for the preparation of β-myrcene, but in no case was any evidence found of the presence of this material, which should be so easily recognizable on account of its high refractive index. None of the products underwent polymerization when treated with sodium and barium peroxide as directed by Ostromislenski, and none reacted with sulphur dioxide solution except apparently in so far as some of the fractions were contaminated with isoprene. The iodine absorption of one of the products was determined and found to correspond to the presence of two double bonds only. Further, the oil obtained in one experiment was subjected to bromination and proved to contain a considerable proportion of dipentene.

The temperatures at which isoprene was polymerized were (a) room temperature, (b) 85-92° C., (c) 145° C. Polymerization was also carried out at 90° C. in the presence of benzoyl peroxide. The physical properties of the main fractions are shown in Table IV.

A definitive investigation of the dimeric polymerizate from isoprene probably demands the careful fractionation and study of a much larger quantity of material than has been employed by any workers hitherto. In the opinion of the present authors the material is preponderantly dipentene. The fact that on bromination it yields a considerable amount of oily bromide in addition to

Four litres of crude isoprene prepared by the lamp was dried over calcium chloride and fractionated several times. The method of purification at first employed was one described by Ostromislenski (26). Isoprene (1500 cc.) boiling at 33-36° C. was allowed to stand at room temperature over 16 gm. of sodium wire and 135 gm. of barium peroxide for at least two days; it was then refluxed for one hour and fractionated. This gave 1300 cc. of isoprene boiling at 33-35° C. As it appeared in the first experiment (Experiment A) on polymerization, that the above method does not yield isoprene of very high purity, the material used in all but the first experiment was subjected to further purification involving conversion to the tetrabromide*.

Following the procedure of Gustavson (7) 100 cc. of isoprene, dissolved in 100 cc. of chloroform, was introduced into a two-litre flask fitted with a reflux condenser, dropping funnel and mechanical stirrer. The flask was cooled in a bath to −10° C. and a previously cooled solution of 320 gm. of bromine in 500 cc. of chloroform was added drop by drop. It was found after five or six experiments that these concentrations were the most convenient. In more concentrated solutions a certain amount of hydrobromic acid was evolved. The solution of bromides thus obtained was washed with aqueous soda solution until colorless and the chloroform layer was dried over anhydrous potassium carbonate. The chloroform was distilled off and the concentrate, generally brown in color, distilled *in vacuo*. The three main fractions were as follows:— I. 60-70° C. at 12 mm. This, containing amylene bromides, was discarded. II. 88-94° C. Isoprene dibromide. III. 155-160° C. Isoprene tetrabromide. The yield of the second and third fractions was about 75-80%. After three or four distillations *in vacuo*, colorless, or almost colorless, products were obtained. As they turned dark on standing, they were used at once. Only a small proportion of the dibromide was obtained.

The regeneration of the isoprene from the tetrabromide was carried out as follows:— 320 gm. of isoprene tetrabromide was added slowly by means of a dropping funnel to 450-500 cc. of alcohol in which was suspended 262 gm. of zinc dust, the latter being added in three portions, and the reaction mixture being kept in constant agitation by an electrically driven stirrer through one neck of the flask (three-necked type). To this flask was attached a reflux condenser kept at a temperature of 45°-50° C. The rest of the apparatus for collecting the isoprene was similar to that in Fig. 3. Traces of alcohol carried over with the isoprene were removed by washing. The yields by this method were 85 to 90% of the theoretical. If stirring was not applied, they were much poorer. The same procedure was applied for the regeneration of the isoprene from the dibromide fraction, the yields being 75-85%.

Finally the regenerated isoprene was dried over calcium chloride and distilled. The distillate was then allowed to stand for several days over sodium wire and redistilled. The boiling point of the pure product was 33.4 to 33.9° C. at 744 mm.

**Cf. References 15 and 24.*

Preparation of 2, 3-Dimethylbutadiene-1, 3

Pinacone hydrate was prepared from carefully dried and redistilled acetone by reduction with amalgamated aluminium as follows. To 200 gm. of 20-mesh aluminium in a perfectly dry three-litre flask a solution of 30 gm. of mercuric chloride in 300 cc. of acetone was added rapidly with agitation. The mixture was left undisturbed for 15-20 min., and then 10 gm. of finely divided zinc chloride and 100 cc. of acetone was added. The mixture was allowed to stand 1½-2 hr., with frequent vigorous agitation, to prevent caking of the aluminium. Then 500 cc. acetone and a solution of 30 gm. of mercuric chloride in 600 cc. of acetone was added dropwise over a period of four hours at such a rate as to keep the liquid boiling, 100 cc. of one and 100 cc. of the other reagent being added alternately. The flask, covered with a cloth, was allowed to stand overnight. Generally it was found refluxing slowly in the morning. In all the reaction took 35-40 hr. Moisture was excluded. Stirring was not found to be necessary so long as the aluminium was kept in a loose condition. Finally the flask was heated on the water bath for two hours. Then 1200 cc. of water was added gradually to decompose the complex, the mixture was boiled for two hours, allowed to cool to 40° C., and filtered. The mass was extracted with four 1000-cc. portions of acetone. The filtrate and washings were concentrated to about 2000 cc. and the supernatant oil removed. The aqueous liquid on standing overnight in an ice box deposited crystals of pinacone hydrate. The oil, which consisted of mesityl oxide, phorone, etc., deposited crystals of pinacone. The total yield of pinacone hydrate was about 350 gm. The product was recrystallized twice by dissolving it in an equal weight of hot water, filtering and cooling in ice. Water of crystallization was removed from the pinacone hydrate by distillation, practically all the water being given up under 135° C. Pinacone carried over to the extent of 10-15% was recovered. A long air condenser and well-cooled receiver were used. Yield: 85-90%. The use of potassium hydroxide and potassium carbonate as dehydrating agents was not as convenient as the method described.

For conversion of pinacone into the diene the following gave the best yields of the methods tried. Pinacone (500 gm.) was distilled with 2 gm. 48% hydrobromic acid (16), half of the pinacone being added through a separating funnel. The reaction proceeded very slowly, but after 24 hr. most of the pinacone had reacted. The hydrocarbon layer of the distillate gave after one fractionation 55% of the theoretical yield of dimethylbutadiene boiling from 68 to 72° C. By repeated fractionation of the higher boiling fractions a further quantity of diene was recovered, the total yield from 3000 gm. of anhydrous pinacone being 1600 gm. of dimethylbutadiene (75%).

Conversion of pinacone to the diene by means of potash alum, by means of sulphanilic acid—methods used by Macallum and Whitby (23)—or by means of potassium bisulphate did not give as good yields as the procedure just described.

Impurities in the diene, mainly pinacoline, were removed by treating the carefully dried and fractionated product with sodium. After several such

treatments, the final product had—b.p. 69.5-70.5° C.; n_D^{22}, 1.4370 (one sample); n_D^{24}, 1.4367 (another sample). Kondakov gives n_D^{20} 1.4375; and Macallum and Whitby (22), n_D^{20} 1.4376.

Polymerization of Isoprene

Examination of the Oily By-products

Experiment A. Following a procedure of Ostromislenski (28) for the preparation of "β-myrcene", 200 gm. of isoprene was heated in an autoclave for five days at a temperature ranging from 85 to 95° C., but for the most part at 92° C. Unchanged isoprene (56% of the total) was removed from the resulting colorless, syrupy liquid by distillation from a water bath. The residue was then distilled with steam and gave 65 cc. (27% of the isoprene used) of a clear, colorless oil and 30 gm. (15%) of a rubber-like residue.

The oil, which had a peculiar odor unlike that of a stock sample of dipentene, was dried in ether over anhydrous sodium sulphate, and then distilled under 10.5 mm. It distilled over between 53.5 and 56.5° C., leaving a small residue of very viscous, colored oil. The residue when treated with alcohol gave a fine crystalline precipitate, the nature of which was not investigated.

Examination of the distillate gave the following results:— mol. wt.. (cryoscopically, in benzene), 139.9 (calcd. for $C_{10}H_{16}$: 136.2); $n_D^{14.5}$, 1.4769. (A sample of dipentene gave $n_D^{14.5}$, 1.4765.) It gave only a trace of amorphous,

TABLE V

Iodine absorption, by Hanus' solution, of oil obtained in Experiment A

Period of absorption (hr.)	5	5.5	7.5	24
No. of double bonds	1.8	1.8	1.9	2.1

white precipitate when treated, even for several days, with a concentrated aqueous solution of sulphur dioxide. When heated according to Ostromislenski's instructions (28) with sodium and barium peroxide in a glass tube for three days at 100° C., it gave no evidence of the occurrence of polymerization.

Distillation under atmospheric pressure yielded the following fractions. There was no sign of polymerization during this distillation.

Fraction 1, up to 172° C. (corr.). Fraction 2, 172-174° C.; n_D 16.5° C., 1.4750. Fraction 3, 174-176° C.; n_D 16.5° C., 1.4750. Fraction 4, 176° C.-end.

Fractions 2 and 3, which comprised most of the oil, were shown by bromination to contain a considerable proportion of dipentene. On distilling a sample of dipentene most of it came over between 174 and 176° C. (b.p. of pure dipentene is given as 175-6° C.). As Fraction 2 above was equal in volume to Fraction 3, it appears that the oil from isoprene contains one or more other cyclic dimers in addition to dipentene. The bromination results are also in agreement with this assumption.

Samples of Fractions 2 and 3 and also of dipentene were diluted with four parts of absolute alcohol and four parts of absolute ether and saturated in the

cold with bromine. They were allowed to stand in an ice box for two weeks, and the solutions were then evaporated. The residue consisted in each case of crystals mixed with a very viscid oil, but from dipentene the crystals formed a greater proportion of the whole than from the fractions of the other oil. After several recrystallizations from ether each of the batches of crystals melted at 125° C.

Experiment B. Since the isoprene used in Experiment A had been purified only by means of sodium and barium peroxide, and since an iron autoclave had been used, the experiment was repeated using sealed glass test tubes and highly purified isoprene (from the tetrabromide). Pure isoprene (300 cc.) was heated in six sealed glass tubes at 85° C. for 98 hr., all the details given by Ostromislenski in one place* for the preparation of β-myrcene thus being very exactly duplicated. Unchanged isoprene was removed by distillation at atmospheric pressure and the oil was then separated from the rubber-like high polymer by distilling, until no further loss in weight occurred, at a pressure of 9 mm. which was later reduced to 1 to 2 mm. The distillate was collected in a flask cooled in solid carbon dioxide and ether. The oil, amounting to 19.5 gm., was dried over calcium chloride and again distilled under a pressure of 7 mm. Only a very slight residue remained. Re-fractionation of the oil gave the following results at 7 mm. Hg. Fraction 1: 7.3 gm.; b.p., up to 53°/7 mm., mostly at 52-53° C.; n_D^{25} 1.4540. Fraction 2: 11.4 gm.; b.p., 53-54° C./7 mm.; n_D^{25}, 1.4713. Fraction 3: 0.75 gm., residue. The low refractive index of Fraction 1 was shown to be due to the presence of some isoprene.

These fractions were then distilled at atmospheric pressure with the results shown in Table VI.

TABLE VI

PROPERTIES OF FRACTIONS OBTAINED FROM EXPERIMENT B

Fraction	Boiling point °C.	Refractive index at 26° C.	Behavior with an aqueous solution of SO_2
1. Isoprene present	Up to 128		Gave a heavy, white precipitate.
2.	128-171 (mainly 169-171°)	1.4667 to 1.4670	Slight precipitate.
3.	171-174	1.4705 to 1.4710	Very slight precipitate.
4.	174-175	1.4723 to 1.4726	No precipitate.
5. Residue (slightly brown viscid oil)		1.4827 to 1.4820	

The residue was distilled under reduced pressure and a few drops of a clear, colorless oil were obtained; n_D^{25}, 1.4730.

None of the fractions obtained (discarding No. 1) showed any tendency to polymerize when treated with sodium and barium peroxide for three days at 100° C.

Clearly the oil obtained in this experiment was substantially the same as that obtained in Experiment A.

**Cf. Reference 30, p. 94.*

Experiments C and D. In these experiments a higher temperature was applied, isoprene being heated for 5.25 and 12.5 hr. respectively at 145° C. The oil proved to be essentially similar to that obtained in the two preceding experiments, as was shown by the boiling point and refractive index of the fractions into which it was separated.

Experiment E. In a second procedure given by Ostromislenski for the preparation of β-myrcene (27) pure isoprene is heated with 5% of benzoyl peroxide for 10 days at 90° C. Using the same procedure, 50 cc. of pure isoprene and 1.7 gm. of benzoyl peroxide were introduced into each of three Pyrex tubes, sealed and heated as above-mentioned. As in previous experiments, most of the isoprene remained unchanged; after its removal the residue was steam distilled, the oil separated, dried over calcium chloride and distilled under reduced pressure. At 10.5 mm. of mercury the boiling point rose rapidly to 61.5° C. and remained there until the close of the distillation. Two fractions were obtained. Fraction I.: b.p., up to 61.5° C. (mostly at 60-61.5°) at 10.5-11 mm.; n_D^{25}, 1.4710 to 1.4713; D_4^{25}, 0.832. Fraction II.: b.p., 61.5° at 10.5-11 mm.; n_D^{25}, 1.4727; D_4^{25}, 0.838.

The oil fractions behaved in a similar way to those obtained in preceding experiments.

No substance could be isolated in these experiments in any way comparable to the behavior of the so-called "β-myrcene".

Experiment F. About 50 cc. of isoprene was allowed to stand for 4½ years at room temperature in the dark in a bottle of 100-cc. capacity, the space above the liquid being filled with air. At the end of this period no separation of polymer had taken place, but the liquid, although not highly viscous, was noticeably more viscous than the original isoprene. The major portion was distilled at ordinary pressure from a water bath in order to remove unchanged isoprene; the dimeric oil was then removed by distillation at 17 mm., and the residue of crude rubber was weighed: 31.2 gm. gave 0.31 gm. oil (1%) and 5.2 gm. (16.6%) rubber.

The oil, colorless, possessed a peculiar and somewhat ethereal odor. n_D, 1.47125 at 23.2° C., 1.4710 at 23.6° C. It was brominated in solution in absolute alcohol and ether and the solution was allowed to stand in a refrigerator for 24 hr. The crystals which had separated were isolated and after five recrystallizations from ether containing a little alcohol gave a colorless product melting at 123.5-124.5° C. A sample of dipentene tetrabromide prepared in the same way from redistilled dipentene melted at 124.5-125° C. A mixed melting point determination gave 123-124° C.

The crude rubber (5.2 gm.) was dissolved in 40 cc. of benzene and poured dropwise into 200 cc. of absolute alcohol. A white rubbery clot formed. It was removed and dried in a vacuum until constant in weight (1.5875 gm.). When dry it was clear, colorless, elastic but not strong. Data for the molecular weight of this fraction and for the viscosity of its sols are given in Table VII. The mother liquor from the above precipitate was evaporated to 25 cc. and a further quantity (75 cc.) of absolute alcohol was added. This precipitated a

second fraction of rubber (2.3 gm.), which, unlike the first fraction, was yellowish and somewhat sticky. Evaporation of the mother liquor gave a third fraction, also somewhat sticky and noticeably darker in color.

In addition to the isolation of rubber from the 4½-year old isoprene by distillation, rubber was isolated from a portion by precipitation. The old isoprene (3.6578 gm.), treated with 50 cc. of methyl alcohol, yielded a clot of colorless rubber weighing 0.2218 gm. The mother liquor was then evaporated, the residue was taken up in 5 cc. benzene, and more precipitant was added. This gave a further quantity of 0.1496 gm. rubber. The total rubber recovered was thus 10.2%. The mother liquor contained a further fraction which presumably was in such a low state of polymerization that it was difficult to precipitate it.

Effect of Temperature and Time on the Polymerization of Isoprene

After heating pure isoprene at the temperatures and for the periods specified, unchanged monomer was removed by distillation at ordinary pressure; then oily polymeric products were removed by distillation under reduced pressure until the distillation flask suffered no further loss in weight, and the yield of rubber was obtained by weighing the tared flask. The oil was collected in a receiver cooled with solid carbon dioxide and ether. The yield of oil was determined by evaporating the distillate under reduced pressure until no more diene came off. As the speed of polymerization is influenced by the volume of air in contact with the diene, a constant ratio of diene to air was employed in all but one of the experiments at 85° and 145° C. The tubes were of 165-cc. capacity and each contained 50 cc. of isoprene except in the experiment in which a heating period of 101 hr. was applied. In the latter the tubes had a capacity of 90 cc. and contained 30 cc. of diene. The results are given in Table I.

Molecular Weight of Rubber Polymers and Viscosity of Their Sols

The samples of synthetic rubber obtained in the above polymerization experiments were purified by dissolving them in pure anhydrous benzene and precipitating them with absolute alcohol, this procedure being repeated at least twice. After the greater part of the attached liquid had been expressed from the precipitated rubber, the latter was dried for 4-5 days in a desiccator evacuated by means of a Hyvac pump. All measurements were made immediately the samples were dry, as it was observed that even when they were kept in a vacuum of 9-10 mm. oxidation occurred rather rapidly.

Molecular weights were determined cryoscopically in benzene. In preparing the solutions, bottles containing the rubber and benzene were kept in the dark for four days with occasional shaking. Results obtained at different concentrations are shown in Table VII. Average values have been recorded in Table I.

A uniform procedure was followed in the preparation of sols of the samples for viscosity measurements, the samples being treated with 20 cc. of purified benzene in a 50-cc. bottle and kept for eight days in a dark cupboard, with shaking once a day. Measurements were made with an Ostwald viscosimeter at $30.1° \pm 0.02°$ C.

TABLE VII

ISOPRENE CAOUTCHOUC

Sample, time and temp. of polymerization	Viscosity		Molecular weight	
	Conc., gm./20 cc. benzene	Relative viscosity, benzene = 1	Conc., gm./20 cc. benzene	Mol. wt.
(a) 97.5 hr. at 85° C.	0.1290	1.54	0.2627	3348
	0.2025	1.81	0.6808	4870
	0.4710	3.10	0.6810	4893
	0.4997	3.24	0.6862	5158
	0.6810	4.62	0.8292	4680
	0.6862	4.66		(Mean:
	0.7285	4.79		4589)
	0.8292	5.92		
	0.9316	6.44		
(b) 900 hr. at 85° C.	0.1077	2.01		
	0.2946	3.92		
	0.6830	8.20	0.6830	5562
	0.7004	9.43	0.7004	5867
	0.8098	8.84*		(Mean:
	0.9223	11.85		5715)
(c) 5.25 hr. at 145° C.	0.0715	1.14		
	0.2406	1.57	0.2406	2713
	0.5078	2.36		
	0.6681	3.09		
	0.6935	3.14	0.6935	3630
	0.7380	3.20	0.7380	3360
	0.9325	4.14	0.9325	3460
				(Mean:
				3291)
(d) 12.5 hr. at 145° C.	0.2317	1.97	0.2317	2613
	0.4440	3.03	0.4440	3617
	0.6947	4.65		
	0.6982	4.69	0.6982	4761
	0.8268	5.78	0.8268	4753
				(Mean:
				3936)
(e) 1 year at 10° C. ±	0.0512	1.24		
	0.1172	1.60		
	0.2912	2.57		
	0.4920	6.13		
(f) 4½ years at room temp.	0.2907	5.0	0.4915	2308
	0.4915	7.9	0.6797	2315
	0.6797	13.4		(Mean:
				2312)

**Measurement after 10 days.*

Cold, Catalytic Polymerization of Isoprene and Dimethylbutadiene

To quantities of 0.5 cc. of isoprene a number of halides were added and the following observations made.

(*a*) *Stannic chloride* (2 drops). Polymerization complete in three hours yielding a clear, soft, amber colored, non-elastic mass, which when warmed showed elastic properties if squeezed, and which swelled but did not dissolve in benzene. Addition of alcohol converted it to a white powder.

(*b*) *Antimony pentachloride.*—Two drops of undiluted reagent produced at ordinary temperature a violent reaction, forming a pitchy product soluble in benzene. At the temperature of solid carbon dioxide, the result was similar to that produced by stannic chloride at room temperature. Five drops of a 20% solution in chloroform had a similar effect, the product being orange colored.

(*c*) *Antimony trichloride.*—The powdered reagent produced at room temperature a violent reaction with charring; at the temperature of solid carbon dioxide, a result similar to that with stannic chloride.

(*d*) *Ferric chloride.*—Only slight effect after two days.

(*e*) *Ferric bromide.*—Gave a dark brown weak gel after 12 hr.

(*f*) *Aluminium chloride.*—Complete polymerization after 24 hr. to a yellow flaky mass only slightly soluble in benzene.

(*g-i*) *Thorium bromide*, 5 drops boron trichloride in 20% chloroform solution, 5 drops boron tribromide in 20% chloroform solution.—Little or no evidence of polymerization after 24 hr. in any of the three cases. Addition of alcohol after 48 hr. precipitated a small amount of powdery polymer.

When a 3% solution of rubber in benzene was treated with the following catalysts, *viz.*, $SbCl_5$, $SnCl_4$, $SbCl_3$, $AlCl_3$, $FeBr_3$, highly colored complexes were formed, which on the addition of alcohol yielded white or pale-yellow powders, mostly only very slightly soluble.

Similar results to those obtained with isoprene were obtained by adding antimony pentachloride and stannic chloride to dimethylbutadiene.

Polymerization of dimethylbutadiene

Examination of the Oily By-products

In no case did examination of the oily, dimeric polymerization products yield any evidence of the presence of an open-chain dimer analogous to "β-myrcene".

Pure dimethylbutadiene was heated at 85° C. for periods of 101, 120, 154 and 250 hr., the quantity of diene taken being 100 cc. in all cases except the second, where it was 375 cc. Unchanged diene was removed by distillation under ordinary pressure and the oily polymerization products were then isolated by distillation under reduced pressure. The yield of oil from a single experiment was so small that the oil from the four experiments (5-6 cc.) was combined and subjected to examination. It boiled at 80° C./10 mm. and showed n_D^{26}, 1.4775. It gave no precipitate with an aqueous solution of sulphur dioxide. When heated in a sealed tube at 100° C. for four days, it became slightly viscous and colored and the refractive index rose to 1.4910 (26° C.), but there was no evidence of the formation of a caoutchouc, as it gave no precipitate when alcohol was added.

In another experiment 100 cc. of pure dimethylbutadiene was heated at 145° C. for 12.5 hr. and the oily polymer isolated. After purification the oil gave no precipitate with sulphur dioxide solution. The oil yielded a sharply boiling fraction, 198-200° C./748 mm.; n_D^{21}, 1.4799.

The oil obtained in the above experiments is clearly essentially the same as

the dimeric oil (dimethyl dipentene) isolated by Lebedev (17, 21) and by Aschan (1, 3), from the polymerization products of dimethylbutadiene, and by Kondakov (13) and Richards (29) from the decomposition products of dimethylbutadiene caoutchouc, as Table VIII shows.

TABLE VIII

DIMERIC PRODUCTS FROM DIMETHYLBUTADIENE

Substance	Boiling point, °C.	Refractive index (n_D)
Oil from polymerization at 85° C. (*supra*)	Approx. 80 (10 mm.)	1.4775 (25° C.)
Oil from polymerization at 145°C. (*supra*)	198-200 (748 mm.)	1.4799 (21° C.)
Lebedev's compound	205 (750 mm.) 85 (13 mm.)	1.47716 (19.7° C.)
Aschan's compound	200.5-201.3 (760 mm.) 87 (12 mm.)	1.47915
Richards' compound	205 (760 mm.)	1.47786 (25° C.)

Effect of Temperature and Time on the Polymerization

Samples of dimethylbutadiene were heated, in glass tubes sealed at atmospheric pressure, for different periods of time at temperatures of 45°, 85° and 145° C. The yields of oily dimers and of caoutchouc were obtained in a similar way to that used in the corresponding experiments with isoprene. The results have been summarized in Table I.

In all the experiments with dimethylbutadiene recorded in Table I the tubes used were of the same size (105 cc.) and contained the same volume of diene (50 cc.). The total volume in each experiment at 85° and 145° C. was 100 cc.; in the experiment at 45° C., 250 cc., and in the experiment at room temperature, 1000 cc. A further experiment, in which the ratio of air to diene was higher than in the above experiments, showed *inter alia* that the proportion of air to diene influences the rate of polymerization. In this experiment the tubes were 250 cc. in volume and contained 75 cc. of diene. The total volume of diene used was 375 cc. After 120 hr. at 85° C. the yield of caoutchouc polymer was 18.3%.

Change of Refractive Index with Polymerization of Dimethylbutadiene

Samples of 5 cc. of the diene were heated in sealed tubes of 15-cc. capacity for various periods at 85° C. and the refractive index and density of the total product was measured in each case. As the previous experiments have shown that only a small proportion of dimer is formed at 85° C. and that the main polymeric product is methyl rubber, it was possible to calculate the percentage of rubber formed at any time with fair accuracy. According to Macallum and Whitby (22) the refractive index of methyl rubber is 1.5250 (at 20° C.), and that of the monomer from which it was prepared 1.4377 (at 20° C.). The change in refraction for 100% polymerization is thus 0.0873. The monomer used in the experiments recorded in Table IX had n_D^{25} 1.4637.

In the following experiment the effect of air on the rate of polymerization was again observed. A quantity (10 cc.) of dimethylbutadiene was sealed in

TABLE IX

CHANGE IN REFRACTIVE INDEX AND DENSITY WITH THE POLYMERIZATION OF DIMETHYLBUTADIENE

Time at 85° C., hr.	Refractive index, n_D^{25}	Density, d_4^{25}	Rubber formed %
0	1.4367		
12	1.4420	0.7335	
24	1.4435	0.7372	
72	1.4464	0.7397	8.3 (found)
127	1.4486	0.7457	11.2 (found)
186	1.4500	0.7469	
372	1.4540	Too viscous	19.82 (calcd.)
550	1.4590	Too viscous	25.5 (calcd.)
578	1.4590	Too viscous	

each of two tubes of 25 cc. capacity. One tube was sealed in the ordinary way and the other while the tube was cooled with solid carbon dioxide and ether and it was evacuated to 10 mm. The two were heated for 46 hr. at 85° C. The refractive index of the contents was measured, with the following results.

n_D^{26} of liquid in tube filled with air, 1.4440.

n_D^{26} of liquid in tube sealed under reduced pressure, 1.4420.

n_D^{25} of original diene, 1.4367.

Heterogeneity of Dimethylbutadiene Caoutchouc

Methyl rubber (5.5 gm.) was dissolved in 50 cc. of pure benzene, and there was added a mixture of 80 parts of absolute alcohol and 20 parts of benzene until a faint turbidity appeared. On standing overnight, the mixture separated into two layers, the upper one being quite fluid and the lower one very viscid. The layers were separated and the polymer precipitated from each by the addition of absolute alcohol. This gave Fraction 1 and Fraction 2, the latter being the larger. The products were carefully dried *in vacuo*, and the viscosity of a sol prepared from a portion of each was measured. The balance of Fraction 2 was redissolved in benzene and separated into two sub-fractions (2a, 2b) by precipitation with the alcohol-benzene mixture. The viscosity of sols prepared from the fractions was measured. The results are shown in Table X.

TABLE X

TIME OF FLOW OF SOLS (0.8544 GM./25 CC. BENZENE) OF FRACTIONS OF METHYL RUBBER)

Fraction No.	1	2	2a	2b
Time of flow, sec.	55.4	72.1	67.8	81.3

Unsaturation of Dimethylbutadiene Caoutchouc

The polymer prepared by heating dimethylbutadiene at 85° C. for 101 hr. was purified by repeated solution in benzene and precipitation with alcohol and finally by prolonged drying *in vacuo*. The unsaturation of the product was

TABLE XI

VISCOSITY AND MOLECULAR WEIGHT OF DIMETHYLBUTADIENE CAOUTCHOUC SAMPLES

Sample, time and temp. of polymerization	Viscosity		Molecular Weight	
	Conc., gm./20 cc. benzene	Relative viscosity, benzene = 1	Conc., gm./20 cc. benzene	Mol. wt.
(a) 101 hr. at 85° C.	0.0862	1.24	0.2121	1443
	0.1881	1.53	0.5042	2110
	0.3231	1.96	0.7700	3400
	0.5161	2.80		Mean,
	0.6677	3.69		2318
(b) 154 hr. at 85° C.	0.0819	1.27	0.2230	1973
	0.2632	1.87	0.6788	3982
	0.3181	2.15	0.6713	4017
	0.4974	2.93	0.8597	4067
	0.6713	3.82		Mean,
	0.6788	3.83		3345
	0.7147	4.11		
	0.8088	4.64		
	0.8597	5.08		
	0.9076	5.36		
(c) 250 hr. at 85° C.	0.2121	1.78	0.2615	2402
	0.3270	2.25	0.6971	4645
	0.4600	3.00		Mean,
	0.6454	3.93		3524
	0.6971	4.55		
	0.8143	5.17		
	0.8990	6.38		
(d) 900 hr. at 85° C.	0.1529	2.49		
	0.2501	3.49		
	0.4830	7.19		
	0.6770	9.21	0.6770	3206
	0.6811	10.86		
	0.8721	13.20	0.8721	3760
				Mean,
				3483
(e) 12.5 hr. at 145° C.	0.2363	1.41	0.6804	2100
	0.4537	1.81	0.6885	2175
	0.6804	2.30		Mean,
	0.6885	2.34		2138
	0.8625	2.84		
(f) 2628 hr. at 45° C.	0.2418	1.53	0.2418	529
	0.4489	2.06		
	0.5255	2.24		
	0.6883	2.76	0.6883	1923
	0.7315	2.79	0.7315	2102
	0.8570	3.05	0.8570	2294
(g) 1920 hr. at room temp.	0.2435	1.31	0.6838	1700
	0.4705	1.62	0.6612	1799
	0.5345	1.72	0.6922	2278
	0.6305	1.87		Mean,
	0.6813	1.99		1926
	0.7477	2.11		
	0.7961	2.23		
	0.9031	2.33		

determined by the method of Kemp (11). The iodine numbers obtained were 309.1, 307.8, 298.6 and 297. These numbers correspond sufficiently well with the calculated value of 309.1 to show that the polymer contains, as nearly as such a method of analysis can decide, one double bond for each (C_6H_{10}) unit.

Viscosity and Molecular Weight of Dimethylbutadiene Caoutchouc Samples

The rubber-like residues left after removing unchanged diene and oily polymers in the above experiments were purified by precipitation as in the case of the corresponding isoprene caoutchouc samples, and determinations were similarly made of the molecular weight of the samples and the viscosity of their sols. The results are shown in Table XI. Mean values for the molecular weights have been recorded in Table I.

Change in Viscosity of Sols on Keeping

It was found that sols of dimethylbutadiene caoutchouc showed a marked decrease in viscosity when kept. In Table XII are recorded measurements made after keeping a number of sols in the dark for various periods, the volume of each sol being 20 cc. and that of the containing bottles 50 cc. (cf. Fig. 2).

TABLE XII

CHANGE IN VISCOSITY OF SOLS OF DIMETHYLBUTADIENE CAOUTCHOUC ON KEEPING

No.	Conc., gm./20 cc. benzene	Time of flow (sec.) at 30.1° C.		
		8 days after preparation	17 days after preparation	33 days after preparation
1	0.8990	155.4	115.0	81.6
2	0.8143	126.2	98.9	73.6
3	0.6454	95.8	78.4	61.6
4	0.4600	73.2	63.4	51.7
5	0.3270	55.0	50.0	43.9
6	0.2121	43.3	41.2	37.7

NOTE:—*Time of flow of pure benzene: 24.4 sec.*

As the above data show, the higher the original viscosity the greater is the decrease; further, the rate of decrease falls as time goes on.

It was found that the change in the viscosity of the sols resulted from a permanent change in the polymer. Each of two sols which had been kept for 53 days was poured into absolute alcohol. As the precipitates would not

TABLE XIII

No.	Conc., gm./20 cc. benzene	Relative viscosity at 30.1° C.		
		(a) Fresh sol.	(b) After keeping 53 days	(c) After recovering rubber from (b) and redissolving it
1	0.8088	4.65	2.38	2.40
2	0.7147	4.23	2.23	2.23

coagulate, the mixture was evaporated at room temperature, and the product, which was sticky and entirely different from the originally tough, elastic material, was made up to the original concentration in fresh benzene. The viscosities of the solutions, measured next day, agreed, not with the initial but with the decreased values, as the figures in Table XIII show.

The fall in viscosity is apparently due to oxidation; it could be prevented by the addition of dimethylaniline, as is shown in Table XIV in which are recorded the results with a sol of dimethylbutadiene caoutchouc (obtained by polymerization at 45° C.) in benzene.

TABLE XIV

	Time of flow, sec.	
	Originally	After 34 days
Without addition	49.2	40.1
With 0.0926 gm. dimethylaniline in 20 cc. sol.	49.2	49.3

The behavior on vulcanization of some of the rubber polymers described herein will be dealt with in a forthcoming paper.

Addendum. There has come to the authors' attention since the above was written a paper by T. Wagner-Jauregg (33) in which also the alleged formation of open-chain dimers on heating isoprene is traversed experimentally, with results in substantial agreement with those reported in the present paper. Following polymerization conditions given by Harries and conditions given by Ostromislenski, no open-chain dimeric product was formed. On heating isoprene at 85-90° C. for 4-5 days the main terpenic fraction had the following properties:— b.p. 170-174° C., $n_D^{21.5}$, 1.4730. It was not polymerizable on heating with sodium. The author regards it as identical with Aschan's diprene, but did not examine it for dipentene. On heating isoprene (160 cc.) with acetic acid (0.8 cc.) at 100° C. for six days the main fraction had the following properties:— b.p. 172-174.5° C., n_D^{17}, 1.4754; d_4^{19}, 0.8454. This material too, which corresponds to Harries' product, is also regarded as being identical with Aschan's diprene. The author remarks that "diprene" is probably a mixture of closely similar hydrocarbons. The author points out that the recent recognition of the frequent occurrence of the isoprene skeleton in nature, no only in terpenes, sesquiterpenes, and rubber, but also in carotinoids, squalene* phytol, bile acids, cholesterol, hop resin acids, confers added interest on the question of the modes of dimerization of isoprene.

Acknowledgment

The authors wish to thank Dr. M. Katz for making the determinations recorded here on the 4½-year-old sample of isoprene.

**Cf. References 35 and 36.*

References

1. Aschan, O. Ber. 57: 1959-1962. 1924.
2. Aschan, O. Ann. 439: 221-232. 1924.
3. Aschan, O. Ann. 461: 1-26. 1928.
4. F. Bayer & Co. Brit. Pat. 17,734 of 1910.
5. Bouchardat, G. Bull. soc. chim. 24 (2): 111-114. 1875.
6. Bruson, H. A., Sebrell, L. B. and Calvert, W. C. Ind. Eng. Chem. 19: 1033-1037. 1927.
7. Gustavson, G. and Demjanoff, N. Chem. Centr. 2: 1345-1346. 1888.
8. Harries, C. D. Ber. 35: 3256-3266. 1902.
9. Harries, C. D. Ann. 383: 157-227. 1911.
10. Harries, C. D. and Gottlob, K. Ann. 383: 228-229. 1911.
11. Kemp, A. R. Ind. Eng. Chem. 19: 531-533. 1927.
12. Kondakov, I. J. prakt. Chem. 64: 109-110. 1901.
13. Kondakov, I. Rev. chim. pure et appl. 1912, 168. (See Dubosc, A. and Luttringer, A. Rubber: its production, chemistry and synthesis in the light of recent research. Griffin & Co., London. 1918. p. 333).
14. Kondakov, I. Annales de l'Université de Jergew, 1900-1902. (See Dubosc. A. and Luttringer, A. Rubber: its production, chemistry and synthesis in the light of recent research. Griffin & Co., London. 1918. p. 332).
15. Kondakov, I. Le Caoutchouc, 15: 9466. 1918.
16. Kyriakides, L. P. J. Am. Chem. Soc. 36: 987-1005. 1914.
17. Lebedev, S. V. J. Russ. Phys. Chem. Soc. 41: 1868. 1909.
18. Lebedev, S. V. J. Russ. Phys. Chem. Soc. 42: 949-960. 1910. (Chem. Centr. 2: 1744. 1910).
19. Lebedev, S. V. J. Russ. Phys. Chem. Soc. 45: 1249-1295. 1913. (Chem. Abstr. 9: 798. 1915).
20. Lebedev, S. V. J. Russ. Phys. Chem. Soc. 45: 1296-1331. 1913.
21. Lebedev, S. V. and Skavronskaia, N. A. Chem. Centr. 1: 1002. 1918.
22. Macallum, A. D. and Whitby, G. S. Trans. Roy. Soc. Can. 18:III: 191-193. 1924.
23. Macallum, A. D. and Whitby, G. S. Trans. Roy. Soc. Can. 22:III:34-44. 1928.
24. Mokiewsky, W. Chem. Centr. 1: 589. 1899.
25. Muntwyler, O. Thesis, Zurich, 1917. (See Schotz, S. O. Synthetic rubber. E. Benn, London. 1926, p. 94)
26. Ostromislenski, I. I. Ger. Pat. 276,185, March 29, 1913.
27. Ostromislenski, I. I. Fr. Pat. 475,565. July 22, 1914. (See J. Soc. Chem. Ind. 35: 130. 1916).
28. Ostromislenski, I. I. J. Russ. Phys. Chem. Soc. 47: 1911-17; 1928-31. 1915.
29. Richards, A. H. Compt. rend. 153: 116-120. 1911.
30. Schotz, S. P. Synthetic rubber. E. Benn. London. 1926.
31. Staudinger, H. Ber. 59: 3019-3043. 1926.
32. Tilden, W. A. J. Chem. Soc. 45: 410-420. 1884.
33. Wagner-Jauregg, T. Ann. 488: 176-185. 1931.
34. Wallach, O. Ann. 227: 277-302. 1885.
35. Whitby, G. S. Trans. Inst. Rubber Ind. 5: 184-195. 1929.
36. Whitby, G. S. Trans. Inst. Rubber Ind. 6: 40-62. 1930.
37. Whitby, G. S. and Katz, M. J. Am. Chem. Soc. 50: 1160-1171. 1928.
38. Whitby, G. S. and Katz, M. Can. J. Research, 4: 344-360. 1930.
39. Whitby, G. S. and Katz, M. Can. J. Research, forthcoming publication.
40. Whitby, G. S., McNally, J. G. and Gallay, W. Trans. Roy. Soc. Can. 22:III:27-32. 1928.